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A Diurnal Rhythm in the Excretion of Urinary Ketosteroids by Young Men[1]

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THE PRESENCE of a diurnal cycle in the urinary output of 17-ketosteroids might have some consequence in consideration of their functional significance. The marked quantitative changes in excretion accompanying various pathologic states (1, 2) may indicate that the processes involving ketosteroid secretion may be labile to ordinary daily stresses.

SUBJECTS AND METHODS

Seven young men ranging in age from 22 to 29 years undertook the collection of consecutive night and day urine specimens for periods ranging from 5 to 9 days. In most cases the attempt was made to collect 4 specimens for each 24-hour period, but this was not always possible.

Toluol was added as preservative and the urines hydrolyzed and extracted within 48 hours of collection. Our usual method of acid hydrolysis and ether extraction was practiced (3). The dried neutral fractions of the ether extract were separated into ketonic and non-ketonic fractions by the micro-Girard reaction (3). Determinations of the 17-ketosteroid titer were made upon the ketonic fractions by the aqueous alcohol technique previously described (3) except that the aliquots were incubated in the dark for one hour, instead of 45 minutes. All results are expressed in terms of mg. equivalent of 17-ketosteroid per hour. 'Night' values are for specimens representing the period of sleep, ranging from 8 to 12 hours; 'day' values are the means for the total waking hours.

RESULTS

In table 1 are presented the data on the mean day and night values for 17-ketosteroids and the corresponding urine volume outputs. It is evident from the data that *a*) the night values for 17-ketosteroid output are consistently lower than the day values for all subjects; *b*) each individual tends to have a characteristic level of 17-ketosteroid excretion for both night and day; *c*) there is a rough, but far from regular, tendency for the level of 17-ketosteroid excretion to parallel the urine volume output.

In figure 1 are presented graphically the data on specimens taken regularly during the collection period for two subjects (*B* and *D*). The data on the last day's collection for *subject*

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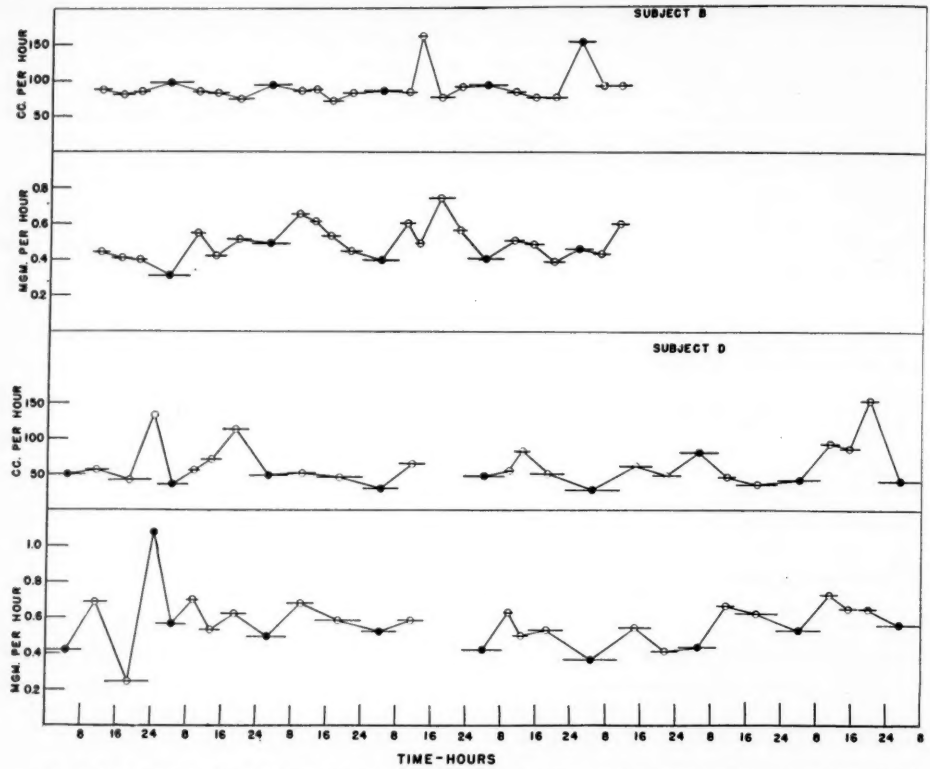

Fig. 1. Data on the rate of 17-ketosteroid and urine output for two subjects. The horizontal bars indicate the time of collection which each urine specimen represents. Time is given on a 24-hour clock, in which 24 is midnight.

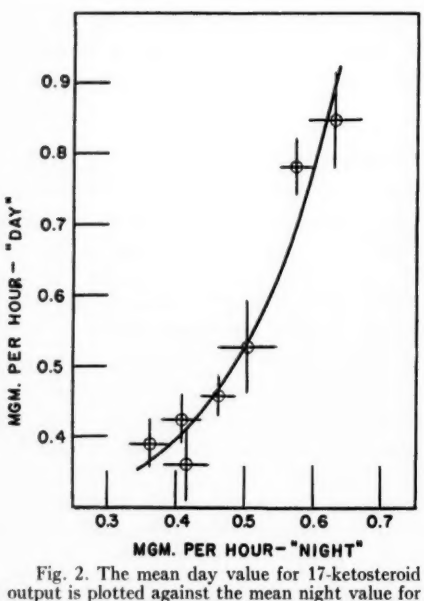

Fig. 2. The mean day value for 17-ketosteroid output is plotted against the mean night value for each of the 7 subjects. The horizontal bars represent ± the standard error for the night values, the vertical bars ± the standard error for the day values. See table 1.

D are omitted. Inspection of this figure reveals *a*) the tendency for 17-ketosteroid excretion to be at a minimum in the specimens taken at night; *b*) a tendency for the maximum 17-ketosteroid excretion to occur in the morning hours. The fourth specimen taken from *subject D* covered a period of a little over an hour when the subject was wakened and asked to take an emergency call on a hospital ward; the high level of both 17-ketosteroid and urine volume output should be noted. Data on the five other subjects exhibit the same general diurnal fluctuations.

On figure 2 the relation between mean 17-ketosteroid output during the day is plotted against the mean night value. These data indicate the possibility of a regular relationship between day and night excretion levels.

The rough parallelism between urine volume and 17-ketosteroid output led to the test of the possibility that the 17-ketosteroids increase in amount during the day simply by being flushed out of the kidneys. *Subject D* undertook a forced diuresis experiment, the data of which are presented graphically in figure 3. After two days of control collections, he ingested, over a period of 3.5 hours, 6.2 liters of fluid of which 5.0 liters was water, 0.6 was soup and 0.6 milk, the soup and milk being taken at the end of the ingestion period. The diuresis ensuing was quite marked, but no significant increase in 17-ketosteroid excretion occurred during the period of diuresis. One specimen, in fact, gave an abnormally low value; this was taken during a brief period following mild signs of water intoxication. At approximately 24 hours after the period of high water intake the subject was called upon for emergency hospital duty which

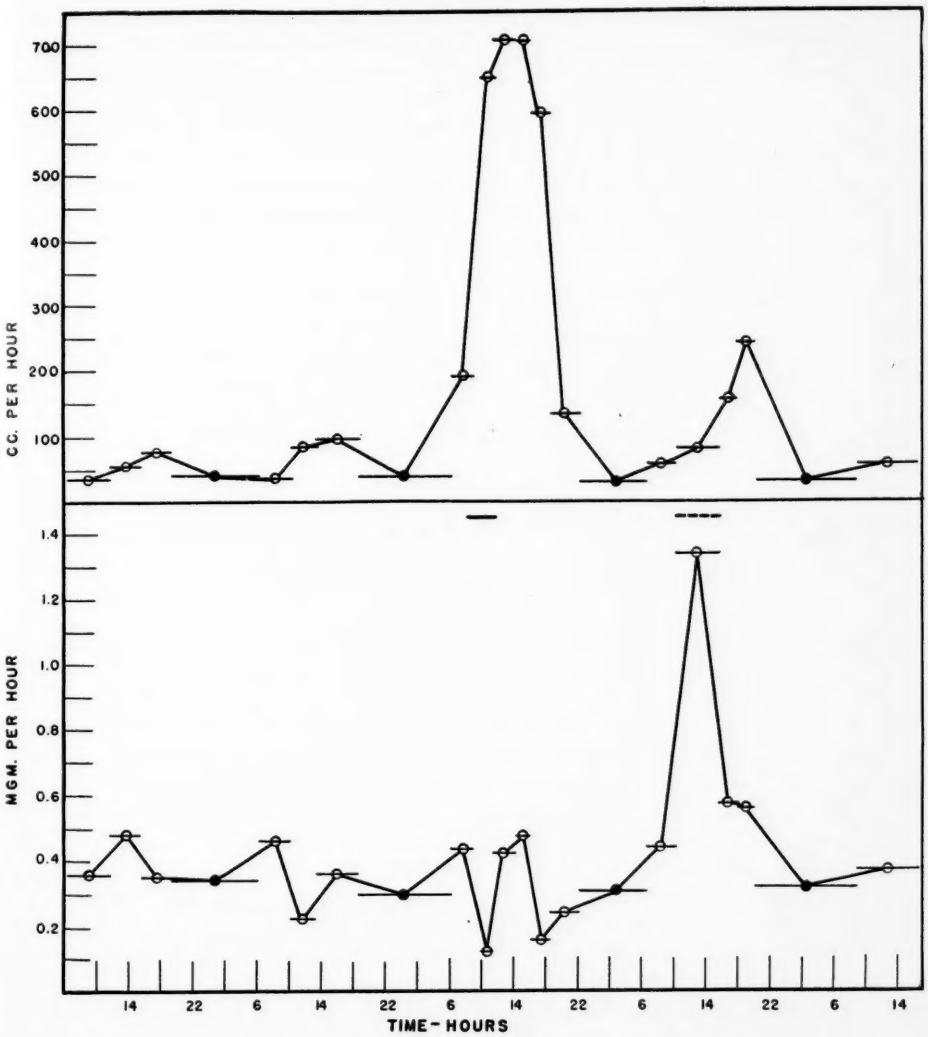

Fig. 3. A forced diuresis experiment on *subject D*. Data on 4 days' collections are plotted as in figure 1. The solid bar represents the period of ingestion of 6.2 liters of fluid, the broken bar a period of emergency hospital duty (see text). Time is given on a 24-hour clock in which 24 is midnight.

lasted through his lunch hour well into the afternoon. The specimen covering this 7.5-hour period gave an unusually high 17-ketosteroid titer, approximately 2.5 times that usually obtained during that time of day. The two succeeding specimens showed slightly elevated 17-ketosteroid titers and increased urine output.

DISCUSSION

Five of the subjects (*A*, *B*, *D*, *F* and *G*) exhibit a statistically significant difference between the night and day values of 17-ketosteroid excretion (table 1), and the data of two subjects (*C* and *E*) in which the lowered night excretion is not statistically significant exhibit the same trend. It is obvious, therefore, that the data as a whole indicate overwhelmingly an increased 17-ketosteroid output during the waking hours. Although Bachman, *et al.* (4), were unable to detect a statistically significant difference in 17-ketosteroid output between successive 12-hour specimens in two women, it should be pointed out that their data do not necessarily apply to men and that their collections were made at 8 A.M. and 8 P.M., respectively, presumably covering in each case a number of waking hours. The rise occurring in the 'day' excretion of these subjects would therefore indicate the need of caution in the use of short-interval collections of urine for clinical routine studies. The night values of 17-ketosteroids for all of these subjects exhibit consistently a lower standard deviation (see table 1) than do day collections and night specimens may therefore be more reliable in setting up a standard.

The data of this paper may be interpreted to indicate that the stress of daily duties tends to elevate the 17-ketosteroid output. Alternatively, we may attribute the increase simply to activity. On two occasions, emergency calls not involving excessive physical exertion induced extreme increases in output; this may indicate tension rather than activity as the cause of the ketosteroiduria and the accompanying diuresis. Since no special control of water intake was practiced by these subjects, the exact relation between urine volume and 17-ketosteroid output cannot be defined. Nonetheless, the forced diuresis experiment indicates that diuresis, *per se*, is not a cause of ketosteroiduria. Bachman, *et al.* (4), found similar indications of the independence of urine volume and 17-ketosteroid output in women.

TABLE 1. THE MEAN DAY AND NIGHT VALUES OF 17-KETOSTEROID AND URINE VOLUME OUTPUTS FOR 7 SUBJECTS[1]

Subject	No. of 24-Hour Periods	17-Ketosteroid Titer, mg. per hour		Urine Volume Output, cc. per hour	
		Night	Day	Night	Day
A	7	0.362±0.029	0.489±0.033	27.2±2.7	47.2± 1.9
B	6	0.409±0.030	0.523±0.035	42.9±1.9	37.5± 3.9
C	6	0.416±0.033	0.460±0.051	22.4±3.0	20.9± 2.5
D	9	0.462±0.026	0.557±0.029	42.4±3.0	62.2± 6.8
E	5	0.504±0.042	0.627±0.065	39.6±8.9	53.4± 6.7
F	7	0.574±0.024	0.882±0.040	33.0±2.3	73.1±11.5
G	8	0.630±0.037	0.948±0.068	44.8±4.1	60.0± 3.1

[1] The standard errors indicated are calculated by the formula: S.E. $= \pm\sqrt{\Sigma d^2 f/n(n-1)}$.

The fact that, over the periods studied, each individual exhibits a characteristic level of 17-ketosteroid excretion deserves more than passing attention. The differences between the means for the night specimens for *subject A* and *subjects D* to *G* are, for example, statistically significant. The day values are more variable, but the mean values for *subjects F* and *G* differ significantly from those of *subjects A* to *E*. We have been unable to correlate age, weight or physical conformation with the observed values. The relative constancy of individual excretion levels has been noted by Fraser, *et al.*, (1) and by Werner (5), but has not been statistically defined by these authors. In view of the fact that their data are derived from determinations made upon the total neutral fractions and not upon the ketonic neutral fractions of urine, comparisons are difficult.

Superficially, the only resemblances between

our subjects excreting 17-ketosteroid at comparable rates seem to involve considerations of temperament and nervous tension. The probability that the 17-ketosteroid excretion is an index of adrenal steroid secretion (1, 6, 7) offers an interesting basis for suggesting a neuro-endocrine mechanism.

SUMMARY

Urine specimens were collected regularly from 7 young men over a total of forty-eight 24-hour periods, and records were made of the 17-ketosteroid and urine-volume outputs on all specimens collected throughout these periods. The night values for 17-ketosteroids are regularly lower than the day values; the maximum day value tends to occur in specimens taken during the morning hours. High night titers appear to be associated with high day titers and vice versa. Forced diuresis did not significantly increase the rate of 17-ketosteroid excretion in one subject; emergency calls in the hospital were associated with significant titer increases in this subject.

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Rapid Clinical Determination of Urinary 17-Ketosteroids

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DETERMINATION of the 17-ketosteroid content of urine specimens has been a lengthy procedure because of the time required for extraction and the detailed process involved in the purification and measurement of the extract. Since the normal variation between individuals is great and since there is considerable change from the normal in clinical conditions in which the test may be used as a diagnostic aid, it seems justifiable to place more emphasis on convenience and speed than on matters of detail that might add slightly to accuracy. The following article describes more fully the method previously outlined by the authors (1). An effort has been made to reduce to a minimum the time required for the determination without a corresponding decrease in accuracy. The resulting method is simple and rapid and yet compares favorably with other published techniques in completeness of recovery and reproducibility of results.

METHOD

The method is used in determining the 17-ketosteroid content of a urine sample.

1. A 24-hour specimen of urine is collected and preserved by adding 10 cc. of concentrated HCl per liter (2).

2. Two hundred and fifty cc. of the urine is placed in a 500-cc. Erlenmeyer flask with a ground-glass connection to a reflux condenser. Twenty-five cc. of concentrated HCl is added and the urine is hydrolyzed by boiling it on an electric hot plate for 10 minutes. A glass bead is dropped in to prevent superheating. There is sometimes a tendency for the hot urine to overflow as it reaches the boiling point, but if the flask is set off the plate for a minute or so at the time boiling starts this can usually be avoided.

3. The flask is cooled slightly and 75 cc. of CCl_4 is added to it. The condenser is attached and the sample is refluxed for 10 minutes in a boiling waterbath on the hot plate.

4. The CCl_4 layer is drawn off by using a separatory funnel and is filtered by suction through one thickness of filter paper in a Büchner funnel.

5. The filtered CCl_4 extract is washed once with about 20 cc. of water, once with 20 cc. of 10 per cent NaOH solution, and twice more with similar amounts of water. This may be done by transferring it back and forth from one separatory funnel to another. It is then placed in a 125-cc. Erlenmeyer flask and evaporated to dryness on a steam bath. A glass bead speeds up this evaporation.

6. Ten cc. of 95 per cent ethanol is added to the flask and the residue is dissolved by slight warming. If the 17-ketosteroid value is abnormally high a greater dilution may be necessary.

7. For the colorimetric determination four 50-cc. test tubes are prepared as follows:

A. Standard solution: 1 cc. of 0.2 mg./cc. dehydroisoandrosterone solution in 95 per cent ethanol.

B. Unknown solution: 1 cc. of the urine extract.

C. Unknown solution blank: 1 cc. of urine extract plus 1 cc. of 95 per cent ethanol.

D. Reagent blank: 1 cc. of 95 per cent ethanol.

One cc. of 2.5M KOH in 100 per cent ethanol is put in every tube, and to each tube, except the one containing the unknown solution blank (*C*), 1 cc. of 2 per cent m-dinitrobenzene in 100 per cent ethanol is added. The alcoholic KOH solution is made fresh each time it is used by grinding

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7 gm. of C.P. KOH in 25 cc. of 100 per cent ethanol, filtering the supernatant fluid to remove insoluble carbonate, adding another 25-cc. portion of alcohol to the residue, which is ground again and then filtered. This grinding is done with a glass rod in a 50-cc. graduate set in ice water. The 2 per cent m-dinitrobenzene solution is made by placing 1 gm. of the reagent in a 50-cc. volumetric flask and filling to the mark with 100 per cent ethanol. The material dissolves more readily if the flask is warmed slightly.

8. The tubes are set in a dark place at about 25° C. and left for 45 minutes. They should be shaken occasionally.

9. Twenty-five cc. of 70 per cent ethanol is added to each tube and the contents mixed by inversion.

10. The light absorbed by each of the preparations is determined in a photoelectric colorimeter with a filter transmitting a peak intensity at about 5200 Å. The instrument is set at zero absorption with a distilled water blank.[1]

11. Calculation: The light absorption value for the urine extract (*B*) is corrected by subtracting the readings for the urine extract blank (*C*) and the reagent blank (*D*) The value for the standard solution (*A*) is corrected by subtracting the value for the reagent blank (*D*) only.

The concentration of the unknown may then be determined as follows:

Concentration of unknown, mg./cc. = 0.2/corrected reading of standard × corrected reading of unknown.

The 24-hour 17-ketosteroid excretion expressed as mg. of dehydroisoandrosterone = concentration of unknown, mg. per cc. × dilution of extract × 24-hour volume of urine in cc./250.

Example:

24-hour volume of urine = 1100 cc.

Urine extract is diluted with 10 cc. 95 per cent ethanol.

Reading of standard solution (*A*) = 55.6

Reading of unknown solution (*B*) = 47.6

Reading of unknown solution blank (*C*) = 2.3

Reading of reagent blank solution (*D*) = 8.6

Concentration of unknown = 0.2/55.6 − 8.6 × (47.6 − 8.6 − 2.3) = 0.16 mg. per cc.

24-hour excretion = 0.16 mg. per cc. × 10 × 1100/250 = 7.0 mg. 17-ketosteroid as dehydroisoandrosterone per 24 hours.

[1] A Fisher electrophotometer equipped with No. 525 filter and 23-cc. cells was used in the determination. Ten readings of a 0.2 mg./cc. dehydroisoandrosterone solution prepared as outlined showed a maximum variation of less than 0.5 per cent.

EXPERIMENTAL RESULTS

The following experimental studies were made to determine the efficiency of the procedure outlined above.

Hydrolysis and extraction. Preliminary hydrolysis before extraction is indicated. Several 24-hour collections of urine from normal males were pooled. Five 250-cc. portions were hydrolyzed by boiling for 10 minutes after adding 25 cc. of concentrated HCl to each. These were extracted by refluxing with 75-cc. quantities of CCl_4 for 5 to 180 minutes. The same amount of acid was added to 5 more 250-cc. samples and these were subjected to a combined hydrolysis and extraction while they were being refluxed with CCl_4 (table 1).

TABLE 1. COMPARISON OF RESULTS FOLLOWING PREVIOUS HYDROLYSIS AND SIMULTANEOUS HYDROLYSIS AND EXTRACTION OF URINE

Minutes Extraction with CCl_4	Recovery After Previous Hydrolysis mg./liter	Recovery with Simultaneous Hydrolysis mg./liter
5	7.1	1.8
15	6.7	4.0
30	6.8	4.2
60	7.8	5.0
180	7.4	6.8

Figures in columns 2 and 3 show amount of 17-ketosteroid recovered expressed as mg. of dehydroisoandrosterone per liter of urine.

The experiment represented in table 2 was performed to measure the efficiency of the CCl_4 extraction. Several daily collections of urine from normal males were pooled and hydrolyzed for 10 minutes with one-tenth volume of concentrated HCl. Four 250-cc. portions were extracted for 10 minutes each and 4 for periods of 4 hours each. The amounts of hormone recovered are shown in columns 1 and 2. The

TABLE 2. EFFICIENCY OF CCl_4 REFLUX METHOD IN EXTRACTION OF 17 KETOSTEROIDS FROM URINE

Ten-Minute Extraction mg./liter	Four-Hour Extraction mg./liter	Re-extraction 10-Minute Sample mg./liter
6.2	6.6	0.6
6.2	6.3	0.7
6.2	6.2	0.6
6.1	6.8	0.6
6.2 mean	6.5 mean	0.6 mean

Figures show hormone extracted as milligrams of dehydroisoandrosterone per liter of urine.

TABLE 3. DEVIATION IN THE AMOUNT OF 1.783 MG. OF TRANSDEHYDROANDROSTERONE RECOVERED FROM 250 CC. OF DISTILLED WATER BY A 10 MINUTE REFLUX PERIOD WITH 75 CC. OF CCl_4

Amount Recovered by CCl_4 Extraction	Percentage Recovery
mg.	%
1.617	90.3
1.654	92.5
1.639	92.0
1.676	93.8
1.631	91.3
1.631	91.3
1.713	96.2
1.649	92.4
	92.5 mean

samples of urine extracted for 10 minutes were then re-extracted for one hour with fresh 75-cc. quantities of CCl_4 and the additional amounts of hormone recovered are shown in column 3.

Table 3 shows another determination of the efficiency of the CCl_4 extraction. Ten-cc. portions of 95 per cent ethanol solution of crystalline trans-dehydroandrosterone were added to 250-cc. quantities of distilled water. The samples of water containing the hormone were refluxed for 10 minutes with 75-cc. amounts of CCl_4. The CCl_4 was then separated from the water, evaporated and the amount of hormone extracted determined as outlined above.

TABLE 4. DEVIATION OF ANALYTICAL RESULTS

Sample 1 mg./250 cc.	Sample 2 mg./250 cc.
1.54	1.45
1.55	1.50
1.56	1.53
1.53	1.49
1.545 mean	1.492 mean
0.97% maximum deviation	2.68% maximum deviation

17-ketosteroids from 250 cc. portions of two urine samples hydrolyzed and refluxed as described in text. The figures represent 17-ketosteroids recovered as mg. of dehydroisoandrosterone.

The preciseness of the determination of the hormone content of identical 250-cc. samples of urine is shown in table 4. Two 1-liter samples of urine of normal males were each divided into 250-cc. portions, then hydrolyzed and extracted as described in the method above.

Washing. The variation involved in the process of washing the CCl_4 extract with 10 per cent NaOH solution and water is indicated by the figures given in table 5. Three hundred cc. of CCl_4 extract from urine from normal males was divided into 4 portions and each of these was washed in separatory funnels with 10 per cent NaOH and water as described in the method.

To determine the effectiveness of a single alkali wash in removing estrogens the following experiment was performed. One 75-cc. portion of CCl_4 extract of urine obtained during the eighth month of pregnancy was washed once with 10 per cent NaOH and 3 times with water. A second identical sample was washed 4 times with NaOH and 3 times with water. A third was washed with $NaHCO_3$, NaOH, and water as recommended by some investigators (3). The amount of hormone determined is shown in table 6.

TABLE 5. DEVIATION IN RESULTS OF WASHING FOUR IDENTICAL PORTIONS OF A CCl_4 EXTRACT WITH WATER AND 10 PER CENT NaOH SOLUTION

17-ketosteroids mg./75 cc. of CCl_4
1.22
1.24
1.25
1.24
1.237 mean
1.4% maximum deviation

When a CCl_4 extract of urine is shaken in a separatory funnel with either water or NaOH solution, there is sometimes a layer of emulsion which may remain at the interface after the layers of solvent and washing solution have separated. This will disappear if the funnel is allowed to stand for a long time, but the amount of solvent saved by this practice is insignificant. Eight 75-cc. urine extracts were washed once with water, once with NaOH and twice more with water, leaving only half a minute or so each time for the two layers to separate. All of the washing solutions used

TABLE 6. EFFECT OF VARIOUS METHODS OF WASHING CCl_4 EXTRACT OF URINE SPECIMEN IN EIGHTH MONTH OF PREGNANCY

Method of Washing			17-Ketosteroid Recovered mg./250 cc. urine
$NaHCO_3$	10% KOH	Water	
	1 time	3 times	1.80
	4 times	3 times	1.64
2 times	4 times	3 times	1.65

were saved and allowed to stand until the solvent left in them had settled out, and this was then measured. For the 600 cc. of CCl_4 washed the amount of CCl_4 recovered from the washings was 6.7 cc., or a loss for each urine extract of only about 1 per cent of the original volume.

Color development. The 1-cc. quantities of standard dehydroisoandrosterone and urine extract solutions are measured with 1-cc. pipettes. The alcoholic KOH and the m-dinitrobenzene solutions do not need to be measured so precisely and a 10-cc. graduated pipette is more convenient. A 25-cc. graduate is used to measure the alcohol for the final dilution. Table 7 lists the electrophotometer readings of 10 identical tubes of standard dehydroisoandrosterone solution prepared as described.

TABLE 7. VARIATION IN ELECTROPHOTOMETER READINGS OF TEN TUBES OF IDENTICALLY PREPARED URINE EXTRACT SOLUTIONS

Electrophotometer Readings		
43.5	43.5	
43.6	43.5	mean=43.94
44.0	44.7	max. deviation=0.80 or 1.8%
44.3	44.3	
44.2	43.8	

Readings are from the logarithmic scale of the Fisher electrophotometer.

If 25 cc. of 95 per cent alcohol is added to the tube containing the reaction mixture there is sometimes a turbidity due to carbonate precipitation. The use of 70 per cent alcohol eliminates this cloudiness and also conserves alcohol. Table 8 gives electrophotometer readings of samples of 0.2 mg./cc. dehydroisoandrosterone solutions that had been allowed to react with alkaline m-dinitrobenzene and were then diluted with 25-cc. quantities of 95 to 55 per cent ethanol. The amount of color is the same for any dilutions of alcohol between 95 and 55 per cent. The amount of fading occurring in a half-hour period after dilution is no greater with the low concentration than with the high.

As a check on the method of color development and determination (4) four portions of an alcoholic solution of urine extract, from 0.25 to 2.0 cc. in volume, were placed in test tubes. The alcohol was then evaporated off, 1 cc. of alcohol was replaced, and the color was developed and measured in the usual way. The 17-ketosteroid determinations are shown in table 9.

TABLE 8. ELECTROPHOTOMETER READINGS OF DEHYDROISOANDROSTERONE SOLUTIONS THAT HAVE BEEN ALLOWED TO REACT WITH ALKALINE *m*-DINITROBENZENE REAGENT AND THEN DILUTED WITH VARIOUS CONCENTRATIONS OF ETHANOL

Concentration of Ethanol Used for Dilution, %	Electrophotometer Readings with No. 525 Filter		
	Extract reading	Reagent blank reading	Corrected extract reading
95	45.3	9.6	35.7
85	44.7	9.0	35.7
75	44.0	8.7	35.3
65	43.9	9.3	34.6
55	43.9	8.3	35.6

Readings are from the logarithmic scale of the Fisher electrophotometer.

DISCUSSION

Simultaneous hydrolysis and extraction with heated CCl_4 has been reported (5), but the data shown in table 1 indicate that even when the urine is kept at the boiling point of CCl_4 the hydrolysis with 10 per cent acid is slow. Three hours of simultaneous hydrolysis and extraction is necessary to recover as much 17-ketosteroid as comes out in 5 minutes with a previous 10-minute hydrolysis at 100° C. When the urine was acidified to 15 per cent HCl and simultaneously refluxed and extracted for 15 minutes the yield was no greater than with 10 per cent acid.

The long extraction period necessary for the removal of 17-ketosteroid from urine has been one of the chief factors that makes the determination time-consuming. Hershberg (6) reported the use of a rapid extractor in which CCl_4 is allowed to drop through a sintered glass disc into a column of urine heated by an electric coil. One-half hour of extraction is reported to be sufficient. The reflux method used

TABLE 9. COLORIMETRIC DETERMINATION OF VARIOUS AMOUNTS OF A SINGLE URINE EXTRACT

Amount of Urine Extract	17-Ketosteroid as Dehydroisoandrosterone	Concentration Ratio
cc.	*mg.*	
0.25	0.033	1.00
0.5	0.069	2.09
1.0	0.134	4.08
2.0	0.272	8.24

by the present authors takes even less time than this. The hot water baths keep the urine at about the boiling point of CCl_4, 76° C., and the solvent makes efficient contact as the droplets rise and fall through the aqueous layer and as the CCl_4 condenses and runs down the sides of the flask.

A reflux method using CCl_4 has been outlined by Sachs and Kurzrok (7) in which the urine is refluxed with three changes of CCl_4 for a 6-hour period, but the process is much longer and no measure of its efficiency is given.

The rapidity with which 17-ketosteroids are removed from urine by the outlined method is indicated by table 2. Four hours of refluxing with CCl_4 resulted in recovery of only 5 per cent more hormone than was obtained by 10-minute extraction. Re-extraction of the 10-minute samples with fresh CCl_4 produced about 10 per cent additional chromogenic material. These figures seem to indicate that at least 90 per cent of the hormone is removed from the urine in the 10-minute extraction period. This is further substantiated by the results presented in table 3, which show that an average of 92.5 per cent of the amount of crystalline hormone added to distilled water can be recovered by a 10-minute extraction. The range for the eight extractions listed was less than ± 4 per cent.

Most investigators have used either a one-liter sample or a full 24-hour urine specimen for 17-ketosteroid determinations. It is not only more convenient to use smaller portions but there is a considerable saving of solvent and acid. Table 4 shows that the hormone content of samples of urine as small as 250 cc. can be measured precisely. In the four determinations on one sample of urine the greatest deviation from the mean value of hormone recovered was less than 3 per cent. For the other sample it was less than 1 per cent.

The variation involved in washing several 75-cc. samples of CCl_4-urine extract is slight enough to be non-significant. This is indicated by the figures in table 5 which show that the greatest deviation from the mean value of four similar determinations was only 1.4 per cent.

Washing urine extracts with a 10 per cent NaOH solution removes certain yellow and brown pigments which may otherwise give high blank readings. Estrogens may interfere with the test if they are not removed because they give a color similar to that of androsterone with the m-dinitrobenzene reagent. Since they are weak phenols the alkali washing takes them out. It seems a loss of time to wash the extract repeatedly for this purpose, however, because the amount of estrogen excreted by women in any 24-hour period is usually too slight to detect by the chemical test. For the whole menstrual cycle the quantity of estrogen excreted is less than 1 mg., and even for the days of highest excretion the value is not much over 0.1 mg. (8). During pregnancy the excretion of estrogen in the urine is greatly increased but even with this greater amount the error possible with only one alkali washing is slight. As shown by the data in table 6 the hormone recovered from a sample of CCl_4 extract of pregnancy urine washed 4 times with alkali is only 9 per cent less than that from one washed once, and some of this loss may have been due to the washing process rather than to the selective removal of estrogen.

It is convenient and rapid to use standard laboratory pipettes for measuring the hormone solutions and reagents in the colorimetric determination. Use of 1-cc. quantities instead of the 0.2-cc. amounts employed by most other investigators makes this possible. Table 7 shows that the electrophotometer readings of 10 identical preparations of a urine extract sample set up in this manner have a mean deviation of less than 1 per cent and a maximum of less than 2 per cent from the average reading.

Some clinical laboratories may not have an accurately controlled 25° C. water bath in which to keep the tubes while the color is developing. Even if this equipment is available there still may be variations in the time required for manipulation of the tubes before they are read in the colorimeter and in the amount of color developed in the reagent blank. It is, therefore, about as convenient and often more accurate, to prepare tubes of standard hormone solution and of reagent blank mixture and make the calculations as outlined in the method, rather than compute the results by interpolation on a previously prepared curve.

Table 9 presents an experimental check of the preciseness of the outlined method for developing color in the hormone solution by the alkaline m-dinitrobenzene reaction. Although the range of concentrations tested was eightfold the maximum variation from the theoretical for any sample was only a ±2 per cent.

Urine contains certain non-ketonic materials that give interfering yellow color. This fraction of the total chromogenic substance is normally slight, 0.3 to 2.4 mg./24 hr. (9) and is usually considerably less than the daily variation. However, if the 17-ketosteroid excretion is low this interfering fraction may make up a large portion of the total. The non-ketonic fraction may be eliminated by means of the Girard reagent (5), but this process adds considerably to the time necessary for the determination, and there is always a loss of 17-ketosteroid involved (sometimes as much as 10 to 15 per cent, 9). It is simpler to make a correction for the yellow-colored fraction by determining the extinction coefficients for the reaction solution with a green and a blue filter and using the Gibson and Evelyn equation as outlined by Talbot (10).

The experimental tests presented show that more than 90 per cent of the hormone present in a urine sample is extracted and determined by the method outlined. When the normal variations in hormone excretion are considered it is evident that this degree of preciseness is sufficient for clinical diagnostic purposes. To illustrate the range of individual differences in 17-ketosteroid output reported in the literature the following values are listed. Fraser, *et al.*, (2), list a range of 5.1 to 14.2 mg./24 hr. with an average of 9.0 mg./24 hr., for normal females, and for males 8.1 to 22.6 mg./24 hr. with an average of 13.8 mg./24 hr. In addition to this, they report a daily variation for individuals that may amount to as much as 5 mg. Werner (9) states that the daily variation observed in both males and females was within ±40 per cent about the mean value. Talbot and Butler (11) declare that the chances are 2 to 1 that a single assay will fall within 15 per cent of the 17-ketosteroid value obtained by averaging 30 or 40 consecutive daily assays. The clinical conditions in which the test may be of use result in values either significantly lower or very much higher than the normal. Fraser, *et al.* (2), report values of 132 to 220 mg./24 hr. for adrenal tumor cases. The same authors found less than 0.5 mg./24 hr. for women with Addison's disease and 1.5 to 6.4 mg./24 hr. (average 2.7 mg./24 hr.) for men. Only 2 of 15 patients with panhypopituitarism showed any detectable 17-ketosteroids. Hypothyroid cases also were very low. It appears that an analysis must show a considerable deviation from the normal to be significant; therefore the preciseness of the determination is of relatively minor importance.

SUMMARY

A rapid method for the extraction and determination of total urinary 17-ketosteroids is presented. The urine is hydrolyzed with 10 per cent HCl, extracted for 10 minutes by refluxing with boiling CCl_4, and the hormone content determined by the alkaline m-dinitrobenzene technique. Tests of the accuracy of the method show a maximum variation of about ±5 per cent for any stage of the process, and an overall variation of ±10 per cent or less. Since the individual and daily variations are considerably in excess of this amount the procedure is satisfactory for clinical use.

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Purification of Equine Gonadotropin and Its Effect on the Appearance of Antigonadotropic Substances in Human Sera[1,2]

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THE PRESENCE of antigonadotropic substances in human sera following treatment with equine gonadotropin (pregnant mare serum) has been demonstrated in men and women (1, 2). Tests for the presence of these antigonadotropic substances in human sera indicated that they were initially present about 10 weeks after treatment was instituted and increased in titer with continued therapy. Furthermore, these antigonadotropic substances remained in the serum for months after cessation of treatment. Therefore, if the equine gonadotropic activity is nullified by the body as is indicated by our tests in mice and rats, then continued treatment with the hormone would be essentially valueless.

Whether the antigonadotropic evocating factor is the actual hormone or the nonhormonal material in the extract remains to be determined with crystalline preparations. However, extracts of different degrees of purity have been investigated and Gordon (3) has recently shown in tests in rabbits and rats, using 4 pregnant mare serum preparations of different degrees of purity, that the antiserum formed to the more purified equine gonadotropin was the more inhibitory. Gordon's results suggest that antihormone formation to equine gonadotropin is evoked by the actual hormone or some chemically related substance. The present study was undertaken to determine whether further purification of equine gonadotropin would influence the appearance of antigonadotropic substances to equine gonadotropin in the human species.

Over a period of 18 months, 21 women, age 17 to 40 years, were treated with equine gonadotropin. Of this group, 3 patients, age 17 to 20 years, were suffering from primary amenorrhea whereas the other patients exhibited secondary amenorrhea or irregular menstrual periods for 8 months to 10 years. In the evaluation of the patient urinalysis, blood count, hemoglobin determination, basal metabolic rate and endometrial biopsy were made. In addition, a serum test for antigonadotropic substances against equine gonadotropin was obtained before the hormone was administered.

One male patient was treated for hypogonadism.

The patients received either of two preparations of equine gonadotropin,[4] one being approximately 2.5 times more highly purified than the other on the basis of total nitrogen. One lot (#18861) contained 0.004 mg. total nitrogen per 20 I.U. (one Cartland-Nelson rat unit) and 2 other lots (#18818, 19487) contained 0.0016 mg. total nitrogen per 20 I.U. The less purified preparation was commercially available and was used clinically.

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[4] The equine gonadotropin, (Gonadogen) used in these studies was generously supplied by Dr. G. F. Cartland of the Upjohn Company, Kalamazoo, Mich. One Cartland-Nelson rat unit is the equivalent of 20 I.U.

TABLE 1. ILLUSTRATION OF DATA OBTAINED IN TESTING FOR ANTIGONADOTROPIC SUBSTANCES TO EQUINE GONADOTROPIN IN HUMAN SERA FROM CASES OF AMENORRHEA

Treatment	No. of Mice	Injections		Ovarian Weight, mg.	Uterine Weight, mg.	Neutralization
		Equine gonadotropin I.U.	Serum, cc.			
None	4	10	0.9	9.8	28.0	negative
Equine gonadotropin	4	10	0.9	5.2	37.0	partial
Equine gonadotropin	3	10	0.9	3.1	33.0	complete
Equine gonadotropin	3	10	0.9	2.5	5.3	complete
Control experiments						
None	152	10	none	9.4	30.7	
None	20	none	none	3.0	6.8	

Patients generally received 4 injections of hormone during the first 9 to 12 days of each month of treatment. Most of the cases were treated for 1 or 2 months, but in 3 cases they were treated for 3 months and 1 case was treated for 4 months. Total dosages of 800 to 9000 I.U. (40 to 450 Cartland-Nelson rat units) of equine gonadotropin were given, dosages of 2000 or 4000 I.U. being the usual amount administered during a single month. If as much as 2000 I.U. was given, the patient received at 2 to 3-day intervals subcutaneous injections of 200, 400 and 600 I.U. and a final injection of 800 I.U. intravenously.

The blood obtained for antigonadotropic tests was allowed to clot, the serum separated and centrifuged and stored in the refrigerator until tested, which was usually 24 to 48 hours after the blood sample was taken. As test animals, 22-day-old female mice were used. All test mice received a total of 10 I.U. of equine gonadotropin and, at a different site, a total of 0.9 cc. of the serum to be tested. Injections were made subcutaneously once daily for 3 days and the animals were killed 72 hours after the first injection. The weights of the ovaries and uteri were obtained, the latter after removal of intra-uterine fluid. At least 3 or 4 mice were used for each serum test and 2 or more mice receiving equine gonadotropin alone, served as controls. The patient's serum was tested against the lot of hormone with which the patient had been treated.

RESULTS

The sera of each of 39 patients were tested for antagonistic substances against equine gonadotropin from pregnant mare serum before hormone treatment was begun. The 21 patients later treated with the hormone are included in this group. In only one case was there a suggestion of partial inhibition of the gonadotropic action. In this case, the ovarian weight for 3 mice treated with equine gonadotropin and serum averaged 5.3 mg. whereas equine gonadotropin alone increased ovarian weight to 9.0 mg. in 2 control animals. However, this patient did not develop antigonadotropic substances when treated with equine gonadotropin. On the other hand, the serum from one patient appeared to augment the response to equine gonadotropin in 3 mice in which the ovarian weight averaged 14.2 mg. as compared with ovaries averaging 9.2 mg. from 2 mice injected with only equine gonadotropin. Considering the group as a whole, 100 mice received sera from 39 individuals plus equine gonadotropin and the ovarian weights averaged 8.9 mg. as compared with the ovarian weight of 9.2 mg. of 57 littermate mice injected with equine gonadotropin alone. It is evident that antagonistic substances to equine gonadotropin are not normally present in the human female.

Serum from the one male patient was negative prior to treatment.

The sera from 22 amenorrheic women obtained before treatment with equine gonadotropin were also tested. Mice were injected with a total of 0.9 cc. of serum alone and in 14 cases no stimulation was obtained, the ovarian weight and uterine weights being 2.5 and 8.0 mg., respectively, which compares with the organ weights of uninjected mice (table 1). The sera from 8 amenorrheic patients caused a definite stimulation of the uteri of the test

mice which averaged 31.4 mg. (20–50 mg., range). The ovaries were not stimulated, weighing 3.2 mg. As little as 0.3 cc. of serum from one patient increased uterine weight to 31.9 mg. Unfortunately, only 2 of these 8 women returned to the clinic. Retests were made in these 2 cases and the serum (alone) was tested in normal and spayed mice but negative results were obtained. The reason for this phenomenon is not clear with the evidence at hand.

The presence of antigonadotropic substances can be determined by a partial or complete suppression of the ovarian weight increase usually produced by a uniform dosage of equine gonadotropin. The antagonistic effect may reach a sufficiently high titer to cause an inhibition of both ovarian and uterine weight increases. An example of these data is shown in table 1. The administration of 10 I.U. of equine gonadotropin increased ovarian weight to the same degree as 5 Cole-Saunders rat units (10 I.U.), the results with the latter amount were given in a previous report (1).

Seven patients were treated with 1200 to 9000 I.U. of preparation #18861, which contained 0.004 mg. total nitrogen per 20 I.U. In 6 patients antigonadotropic substances were first definitely present in the sera 36 to 92 days after the initial injection (av. 63 days). Two additional patients were injected with this preparation but could be studied for only 28 and 42 days after treatment at which time the sera were negative; on the average, this was not sufficient time for the development of antigonadotropins to equine gonadotropin.

Sera tests were made prior to the appearance of antagonists in all but one case (*E.O.*) and at several intervals after they appeared. Table 2 illustrates the time in days after the first injection when the sera tests were made and also the approximate time of administration of additional equine gonadotropin. At 9 months after the start of the injections, the serum of one patient (*E.S.*) remained strongly inhibitory whereas with 4 patients it was possible to determine the approximate time of disappearance of the antigonadotropic substances. These data suggest a period of 3 months after the last injection as the minimum time required for the loss of demonstrable antagonistic substances.

A detailed illustration of the test data obtained from one patient (*P.C.*) is shown in table 3. The serum from this individual com-

TABLE 2. ANTIGONADOTROPIC ACTIVITY OF SERA FROM PATIENTS FOLLOWING TREATMENT WITH THE LESS PURIFIED EQUINE GONADOTROPIN (#18861)

Patient, Age	Sera Tests, Days after First Injection Equine Gonadotropin, I.U.												
E.S., 36	0	24	44	66		165		245	270				
	2000	4000		+	2000	+	1000	+	+				
P.C., 26	0	28	48	63		78		112	125	170	191	247	
	4000	2000	+	+		+	2000	+	+	+			
N.W., 28	0	28	63	84		286							
	4000	4000	+	+									
B.P., 40	0	47	75	145		167		244					
	4000	1000	+	+									
F.C., 24	0	70	92	185									
	2000		+										
E.O., 27	0	36	70										
	2000	+	+										
A.D., 28	0	42	186	214		256							
	1200												

+ = antigonadotropic substances present.

pletely inhibited the ovarian stimulating effect of equine gonadotropin in 6 tests made during a period of 4 months, but at no time was the serum strong enough to inhibit uterine weight increase. It was interesting to us to find that tests for antigonadotropins did not remain positive for a longer period of time in this patient in view of the fact that a third series of injections was given at 91 to 105 days after the first injection. Varied amounts of serum obtained at 78 days after the start of treatment were tested in combination with the uniform dose of equine gonadotropin, the results of which are shown in table 4.

The male patient received equine gonadotropin (#18861) and antigonadotropin substances were observed at 61 days after the initial injection.

The only patient that failed to exhibit antibody formation with the less purified preparation was one to whom the smallest dosage had been administered (1200 I.U.). However, it will be noted in table 2 that we were not able to obtain serum at the more critical period between 42 and 186 days so that it is possible that antihormones could have been detected during this time since the 60 to 70-day period appears to be the more likely time to find first the presence of antagonistic substances.

The more highly purified equine gonadotropin preparations (#18818, 19487) were administered to 12 individuals in total dosages of 1200 to 8000 I.U. Patients were injected with these amounts to permit direct comparison with the effects of the less purified preparation. As shown in table 5, only one (*M.W.*) of the 12 individuals developed antigonadotropic substances in the sera, although 7 women were injected with the 8000 I.U. dosage. In virtually all of these cases, sera tests were made during what might be the anticipated time for antigonadotropic substances to make their appearance. However, it must be pointed out that the one positive case in this series had a serum with the highest antihormone titer of any obtained. The serum (0.3 cc.) not only abolished the increase anticipated in ovarian weight in the test mice but in the uterine weight as well (table 4).

TABLE 3. SERA TESTS IN MICE OF ONE PATIENT (*P.C.*) TREATED WITH THE LESS PURIFIED EQUINE GONADOTROPIN (#18861)

I.U. Injected, days	Time After Initial Injection days	Average Ovarian Weight, mg.		Test
		Equine gonadotropin and serum	Equine gonadotropin only	
4000	0	10.2	8.0	−
(1–10)	28	7.4	9.8	−
2000	48	3.1	9.1	+
(32–42)	63	3.7	12.6	+
2000	78	3.9	10.8	+
(91–105)	112	2.6	9.8	+
	125	2.7	8.8	+
	170	4.7	8.1	+
	191	11.8	11.5	−
	247	12.0	9.2	−

TABLE 4. ANTIGONADOTROPIC ACTION OF VARIED AMOUNTS OF SERUM

No. of Days Treated	No. of Test Mice	Total Dosage		Average Ovarian Weight, mg.	Average Uterine Weight, mg.
		Eq. gonad. I.U.	Serum cc.		
Patient P.C., preparation #18861 administered					
78	3	10	0.9	3.9	34.0
	3	10	0.6	4.5	42.0
	3	10	0.3	6.7	37.0
	3	10	0	10.8	38.0
Patient M.W., preparation #18818 administered					
49	3	10	0.9	2.5	5.3
	3	10	0.3	2.4	6.8
	3	10	0	7.3	34.0
Control tests	20	0	0	3.0	6.8

The data obtained on one case (*E.M.L.*) treated with the more purified preparation of equine gonadotropin are shown in table 6. The absence of any antagonistic substances in the serum of this patient is well illustrated by the very similar ovarian weights of the test mice as compared with the littermate control mice injected at the same time with equine gonadotropin alone.

DISCUSSION

The results in the present investigation are in agreement with previously reported results (1, 2) that antigonadotropic substances will develop in the blood of human beings following treatment with equine gonadotropin from pregnant mare's serum but our data show that with highly purified preparations the incidence is markedly reduced. The antagonistic sub-

TABLE 5. ANTIGONADOTROPIC ACTIVITY OF SERA FROM PATIENTS FOLLOWING TREATMENT WITH THE MORE HIGHLY PURIFIED EQUINE GONADOTROPIN (#19487, 18818)

Patient, Age	Sera Tests, Days after First Injection Equine Gonadotropin, I.U.								
M.G., 36	0 4000	29	42 4000		56	157	199		
M.W., 17	0 4000	31	58 4000		75				
E.M.L., 20	0 4000	31	65 800	3200	79	117	145	201	216
M.S., 17	0 4000	29	56 4000		63				
M.F., 25	0 2000	28	63		84	99			
E.C., 24	0 800	52 400	126 800		175				
L.M.T., 19	0 1200	63	77						
E.L., 34	0 4000	28	63 4000		286				
L.L., 23	0 4000	42 4000	245						
M.W., 18	0 4000	28	42 + 4000		49 +	61 +			
M.G., 34	0 4000	45	57		79	99 800	249	263	
I.P., 23	0 2000	65	93 800		114				

+ = antigonadotropic substance present.

stances may first appear as early as 36 days after the initial injection when as little as 100 R.U. (2000 I.U.) of equine gonadotropin have been administered. A 9- or 10-week period is the more usual time for the initial indication of antigonadotropins, and this time-period corresponds with data obtained from patients treated with another equine gonadotropin preparation (1). Strongly inhibitory serum was obtained for as long as 9 months after the initial injection of equine gonadotropin, the antigonadotropins being present during the last 7 months of this period. After cessation of treatment, a period of at least 3 months is required for the antigonadotropic substances to disappear. One patient treated with equine gonadotropin developed an extremely high titer of antigonadotropins and the serum was tested 4 and 8 months after the last injection. Complete absence of antagonistic substances was evident at 8 months (4).

TABLE 6. SERA TESTS IN MICE ON ONE PATIENT (*E.M.L.*) TREATED WITH THE MORE HIGHLY PURIFIED EQUINE GONADOTROPIN (#19487)

I.U. Injected days	Time After Initial Injection, days	Average Ovarian Weight, mg.		Test
		Equine gonadotropin, sera	Equine gonadotropin	
4000 (1–10)	0	7.1	7.7	–
	31	6.2	7.8	–
4000 (35–59)	65	8.0	8.4	–
	79	9.6	9.9	–
	117	9.0	8.6	–
	145	11.8	11.8	–
	201	8.0	8.2	–
	216	6.7	6.3	–

Pregnant mare serum extract,[4] containing 0.004 mg. of total nitrogen per 20 I.U. developed antibodies in 6 of 7 patients but another preparation, 2.5 times more purified (0.0016 mg. of total nitrogen per 20 I.U.), and used in the same dosage, resulted in antigonadotropin

formation in only one of 12 patients. These data suggest that the antigonadotropic substance is not evocated by the hormone itself, in contrast with the experiments on animals reported by Gordon (3).

Considerable information on this antihormone problem has been reported and the reader is referred to excellent review articles on the subject (5, 6). However, it should be pointed out that comparisons of crude and highly purified preparations of equine gonadotropin as to their ability to develop antihormones have been made in rats, rabbits and monkeys (3, 7, 8). These investigators found the highly purified preparations capable of developing antihormones and, indeed, Gordon's results (3) show that the antagonistic reaction is more intense when sera from animals treated with the highly purified material is used. One can not explain these differences in results by direct comparison because of the difference in species, dosage and equine gonadotropin used. However, the species difference is readily evident and is further exemplified by the recent report of Smith (9) who showed in hypophysectomized monkeys that antibodies to equine gonadotropin are well developed in 20 to 25 days and these substances persist at very high titers for at least 7 months. On the other hand, Jailer and Leathem (1) obtained only a mildly inhibitory serum from patients treated with the same equine gonadotropin when tested 70 days after the start of treatment.

Our results indicate that increased purity of equine gonadotropin will markedly lower the tendency for antigonadotropin formation in the human being, and thus permit the use of larger doses and/or longer periods of therapy in cases in which it is necessary.

The presence of antigonadotropic substances in the blood of normal patients has been reported (10, 11) whereas Zondek and Sulman failed to find antagonistic substances to human chorionic gonadotropin in either normal or amenorrheic women (12). In human sera, Fellows (13) failed to find antigonadotropins or antagonistic substances to gonadotropins from human pituitaries. We tested the sera of 39 amenorrheic women for antigonadotropins of equine gonadotropin and obtained only a suggestion of inhibition in one case. On treatment, this patient did not demonstrate antibody formation. We did find that sera from some patients would increase the uterine weight of test mice.

SUMMARY

Female patients suffering from amenorrhea were treated with either of two preparations of equine gonadotropin derived from pregnant mare serum, one being 2.5 times more highly purified than the other on the basis of their nitrogen content. Six of 7 patients injected with the less purified preparation developed antigonadotropic substances to equine gonadotropin in their blood. This was usually first evident in 9 to 10 weeks after the initial injection and persisted for at least 3 months after cessation of treatment, thus rendering equine gonadotropin therapy essentially valueless. However, with the more highly purified preparation only 1 of 12 patients developed an antigonadotropic serum. These results indicate that increased purification of the equine gonadotropin will markedly lower the tendency for the formation of antigonadotropins in the human.

The sera of 39 amenorrheic women tested prior to hormone therapy showed that antigonadotropins of equine gonadotropin are normally absent.

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A Case of Cushing's Syndrome with Adrenal Cortical Hyperplasia, without Pituitary Basophilic Adenoma or Hyperplasia

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THE CASE reported in this paper has been the subject of two previous reports (1, 2). It seems desirable to conclude the case now in this final paper because the patient had been under observation for a period of 8 years and the diagnosis has been verified by autopsy.

CASE REPORT

The important features, as published in the two previous reports (1, 2) are as follows: *E.R.*, a white unmarried female was first seen at Jefferson Hospital in April, 1934. Irregularity of menses appeared in 1933. The weight had increased, she had noticed growth of hair in her face, dryness of the skin, marked redness of the face, polydipsia, occasional swelling of ankles, defluvium of the hair of the scalp. She presented a typical appearance of Cushing's syndrome with adiposity of the trunk, moon face, ruddy skin, hirsutism and acne. The blood pressure was normal at that time and the glucose tolerance curve was of the diabetic type. No decalcification of the bones was detectable. The sella turcica showed some ballooning in the roentgenogram and the posterior clinoids appeared thinned. In January, 1935, an intravenous pyelogram failed to reveal signs of an adrenal tumor. In October, 1936, she was studied in New Haven Hospital by Dr. Harvey Cushing and was listed by him as a basophilism suspect. A periadrenal air insufflation showed no adrenal tumor.

The patient was treated in the period between 1934 and 1937 with deep irradiation of the pituitary. Following this treatment in June, 1936, the plethoric appearance and the acne of the face disappeared, hirsutism regressed markedly and the patient lost 13 pounds. In October, 1936, she had a menstrual period. Improvement lasted for about one year. When seen in February, 1938, all of the previous signs and symptoms had recurred. Subsequent pituitary irradiation was without effect. In 1939 the disease had progressed further. Blood pressure had risen to 160/120 mm.Hg. The heart was enlarged. In September 1940, she had become progressively weak, the blood pressure was 200/160 mm. Hg and frank diabetes was present. At this time periadrenal air insufflation was performed by Dr. Kenneth Fry and the roentgenogram revealed both adrenals to be enlarged. No tumor could be outlined. In November, 1940, treatment with diethylstilbestrol was started, the dosage being 1 and later 2 mg. per day. This was followed by marked improvement. In spite of continued treatment with diethylstilbestrol this improvement lasted only about 4 months. In February, 1941, diabetes recurred, hirsutism increased, the blood pressure was 160/100 mm.Hg and she again became very weak.

In March, 1941, at the time of the last report (2) she was still in the hospital. The insulin requirement was now 30 U of protamine zinc insulin with a diet of 2000 cal., 70 gm. of protein and 165 gm. of carbohydrate. When she left the hospital in June, 1941, cardiac decompensation had subsided under bedrest and treatment with digitalis and the blood pressure was 175/120 mm.Hg. The diabetes was controlled as outlined above. She was instructed to continue on this regime and to continue taking 2 mg. of diethylstilbestrol daily. She did this under supervision of her family physician. She was seen at regular intervals in the Endocrine Clinic. However, there was no improvement of her condition. In the course of the next months she began complaining of back pain of increasing severity. The ankles started swelling again.

She was readmitted to the hospital on Jan. 6, 1942. The weight loss was now very marked (107 pounds). Edema of the lower extremities was extensive. Blood pressure was 210/170 mm.Hg. Fifty-six to 64 U of regular insulin controlled the diabetes on a diet of 2000 cal., 180 gm. of carbohydrate, and 70 of protein.

Demineralization of the bones had progressed since the time of the last examination (2). Roentgen-ray examination of the spine showed a marked degree of collapse of the seventh, definite narrowing of the ele-

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[1] J. E. Mears Fellow in Medicine and Physiology.

enth and twelfth thoracic vertebrae, marked collapse of the first and beginning collapse of the third and fourth and possibly of the fifth lumbar vertebra. All of these vertebrae as well as the sacrum showed evidence of osteoporosis.

The skull again showed demineralization of the bones, having a somewhat mottled appearance. The sella turcica did not appear to have changed in size or appearance since the time of previous examination in 1940. Serum calcium was 10.6 mg. per cent, phosphorus 3.3 mg. per cent, phosphatase 21.7 Bodansky units. She was given a diet low in calcium for a 3-day test period. The calcium intake for this period was 330 mg. The urinary calcium excretion was 500.7 mg. in the 3 days. During this test period 17.93 gm. of N was excreted in the urine. The N intake was 31.8 gm. No attempt was made to determine the N excretion in the feces.

She continued taking 2 mg. of stilbestrol daily. On Jan. 25, 1942, treatment with testosterone propionate, 25 mg. 3 times weekly by intramuscular injection was started.

Her condition deteriorated rapidly. Several teeth became loose and fell out. She developed an infection of the right ear and several purulent lesions on the face. Bacteriologic culture revealed streptococcus hemolyticus. The temperature rose to 101°F. In spite of treatment with sulfadiazine the infection progressed, cellulitis developed and on January 25 erysipelas of the face was noted. The temperature rose to 102°F. She died on Feb. 25, 1942.

AUTOPSY REPORT

The general external development has been adequately considered above and the description need not be repeated here. Evidence of a rather marked loss in weight, however, was exhibited by the presence of many abdominal striae and the paucity of subcutaneous tissue of the extremities. The right side of the face and the neck showed an extensive purulent cellulitis which had been previously drained by two surgical incisions.

The heart was slightly enlarged, weighing 420 gm. The left ventricle measured 1.8 cm. in thickness and the right 0.6 cm. The aorta showed minimal atherosclerosis. The lungs, other than manifesting some congestion and edema, revealed no gross pathologic changes. The spleen, liver, kidneys and gastro-intestinal tract were essentially normal.

A careful search in the anterior mediastinum failed to reveal any thymic tissue. The thyroid was well encapsulated and on section disclosed no foci. Only two parathyroid glands could be identified. One measured 3 and the other 2 mm. in diameter. The pancreas was not enlarged. Multiple transverse sections, each about 3 mm. in thickness, disclosed several minute hemorrhagic and necrotic foci. The ovaries were flat and atrophic measuring 2 cm. in diameter and 0.6 cm. in thickness. Each contained several white bodies but no corpora lutea. The fallopian tubes were normal. The uterus was not enlarged. Its cavity was of normal shape and size. The endometrium was pink and scanty. The cervix and vagina showed no gross pathologic changes.

Of the skeletal system only the skull, ribs and vertebrae could be examined. The cranium was quite thin and the bones were more brittle than usual. The pituitary gland measured 0.7 cm. superiorly-inferiorly and 2.0 cm. transversely. The inner surface of the pituitary fossa was smooth and glistening. It showed no evidence of erosion. The cortex of both the ribs and the vertebral bodies was thin and brittle. The marrow was friable. The third lumbar vertebra contained a compressed transverse fracture.

The adrenals were markedly enlarged. Each weighed 30 gm. The right adrenal measured 9×6.5×1.5 cm. and the left measured 8×4.5×1.5 cm. The consistency was firm but lacked the hardness of cancerous tissue. Cut surfaces showed a uniform hyperplasia of the cortices. They were orange-yellow, sharply demarcated from the medulla and exhibited no necrotic or hemorrhagic foci. Each adrenal, in addition, contained several small circumscribed, peripheral adenomas measuring up to 0.6 cm. in diameter. These were similar in all aspects to the main cortical tissue.

Microscopic examination. Serial sections of the hypophysis revealed no basophilic adenoma. The cytoplasm of most of the basophilic cells, and of an occasional eosinophilic cell, contained small refractile, sharply defined droplets, some of which possessed granular debris. They were arranged either singly or in clusters and appeared to concentrate around, and sometimes to involve, the nucleus.

Sections of the leptomeninges and the cortices disclosed a diffuse, purulent meningitis with focal areas of metastatic encephalitis.[2]

The normal architecture of the cortex of the adrenals was well preserved. The capsule was of normal thickness and composed of dense fibrous tissue. The immediately subjacent zona glomerulosa was well defined. The cells were arranged in irregular groups and were relatively small. Their cytoplasm was homogeneous and stained a deep pink color. The nuclei were round, vesicular, uniform and for the most part centrally placed. Often two were found within a single cell. The intervening capillaries were filled with blood and were quite conspicuous. The zona fasiculata was exceptionally well preserved. The columns of cells were arranged in a straight radial pattern between which were slender thick and thin walled capillaries. The cells were much larger than those of the zona glomerulosa. The cytoplasm was pink and foamy or vacuolated. Vacuolated cells were particularly abundant in the midportions of the columns. The nuclei were similar to those of the zona glomerulosa zone. They were either centrally placed, or, in the vacuo-

[2] We are indebted to Dr. B. Alpers for the histologic examination of the pituitary gland and the brain.

Dr. L. Eisenhardt of Yale University School of Medicine examined slides of the pituitary gland and found the basophilic cells to show pronounced Crooke changes. We are grateful for her permission to quote this from a personal communication.

lated cells, pushed aside. The zona reticularis was less well defined. The cells were irregularly arranged and some of these were large pale cells, others were smaller and dark. The former resembled the foamy cells of the zona fasiculata while the latter resembled the cells of the zona glomerulosa. In addition, many of the small cells contained in their cytoplasm a sprinkling of fine brown, dust-like granules. The surrounding capillary spaces were quite prominent and filled with blood. The medulla showed no histopathologic changes.

The adenomatous nodules were essentially similar, with the exception that the different zones, although easily recognizable, were less prominent than in those of the adrenal proper.

Sections of the adrenals stained with ponceau-fuchsin as outlined by Uotila (3) were positive for fuchsinophilic granules.

Sections of the pancreas stained with hematoxylin and eosin showed no changes in the islets of Langerhans. Special stains for α and β cells could not be done since the body was autopsied 6 hours after death. The exocrine portion of the pancreas, however, revealed numerous small and large areas of recent necrosis and hemorrhagic extravasation. Acute inflammatory reaction surrounding these foci was minimal. The smaller vessels showed extensive arteriolar sclerosis with occasional complete obliteration of the vascular lumen. In addition to this, several vessels were plugged with antemortem thrombi. The vascular changes in the kidneys were of a similar nature although less severe. There were, in addition, focal areas of arteriolar and glomerular necrosis. The cortices contained several scars and the interstitial tissue showed some edema.

Sections of the vertebral bodies showed atrophic bony spicules with severe hypoplasia of the marrow cells. The parathyroid glands showed a slight fatty infiltration but no histopathologic changes. The ovaries contained a few large follicles and old and young corpora albicantes but no corpora hemorrhagica. The endometrium was of the interval type. The vaginal mucosa was not atrophied. Its superficial cells showed some keratinization while the prickle cells exhibited vacuolar degeneration. Sections from the remaining organs revealed no contributory findings.

Comment. Adrenal glands from several fetuses between 3 and 4 months gestation, from a child 12 years old, from an adult human female and from several adult rats, both normal and castrated, were used as controls for the ponceau-fuchsin stain. The results were so inconsistent that, although fuchsinophilic granules were present in the adrenals of the case under discussion, it could not be stated unequivocally that the cells taking the stain were 'androgenic' cells.

The metastatic encephalitis, the recent vascular thrombi with resulting pancreatic necrosis and the focal arteriolar and glomerular necrosis of the kidneys, probably originated in the acute cellulitis of the right side of the face and neck.

DISCUSSION

This case presented Cushing's syndrome with all its classic features. Autopsy revealed neither tumor of the pituitary gland, nor diffuse hyperplasia of basophil cells, nor tumor of any other gland save several 'knoten' of the adrenal cortex. The measurements of the pituitary gland were slightly above the normal average. There was a marked bilateral cortical hyperplasia of the adrenal which had several small adenomata.

Adrenal cortical hyperplasia is a frequent although not constant finding in cases of Cushing's syndrome due to pituitary basophilism. Eisenhart and Thompson (4) reviewed 67 cases of Cushing's syndrome with pituitary changes and noted adrenal cortical hyperplasia in 42 cases. On the other hand, adrenal cortical hyperplasia in the absence of pituitary basophilism has been found in several instances (5–8).

Crooke-changes of the basophilic cells. Crooke (9) examined the pituitary glands of 12 cases of Cushing's syndrome. Six of these had basophilic adenoma of the pituitary gland. In the other 6 cases no basophilism was found, 3 showing adrenal cortical changes and 3 showing tumors of the thymus. In all cases 'hyalinization' of the basophilic cells of the pituitary gland was found. Eisenhart and Thompson (4) found hyalinization in 40 cases of basophilic adenoma in those basophilic cells which did not constitute the adenoma. There was hyalinization of the basophiles in this case. Crooke considered this change as the causative lesion of the syndrome. Severinghaus and Thompson (10) interpret the hyalinization as a degenerative change following a period of physiologic overactivity. This latter interpretation may be tentatively supported by observations in our case.

Gonadotropic hormone. In an early period of the disease there was a marked increase of gonadotropic hormone in urine and serum on several occasions (2). The first assay report in 1934 reads 'not increased.' This assay was not performed by us and the result reported may

be explained by the inaccurate methods of assay available at that time. Later assay values were always low (table 2, reference 2). This would tend to indicate that there was a period of overactivity of the pituitary gland followed by decreased function.

Adrenocorticotropic hormone. The assays of adrenocorticotropic hormone show the same tendency, increased values followed by a decrease. We should, however, like to make some reservations in quoting these results. Most of the assays were made with serum using the method of Jores (11). For reasons discussed elsewhere (12), we do not consider this method reliable and have abandoned its use entirely. However, the assays did reveal decreasing adrenocorticotropic activity of the serum (2). Assays of adrenocorticotropic hormone in the urine by the method described elsewhere (12) were performed in April, 1941, and again in 1942. Neither urine sample showed adrenocorticotropic activity at this late stage of the disease.

The results of the assays of gonadotropic hormone and, with reservations, also those of adrenocorticotropic hormone, might be interpreted as early hyperpituitarism with subsequent lowering of pituitary function, possibly due to exhaustion of the gland. Some clinical observations in this case would tend further to support such interpretation. The patient improved greatly after deep roentgen-ray irradiation of the pituitary (1). Whether this was a true therapeutic effect or a spontaneous remission coincident with treatment given at that time cannot, of course, be definitely stated. Spontaneous remissions are rare in this disease.

Later, after the relapse in 1938, further irradiation of the pituitary gland was ineffective. It does seem possible that by this time the adrenal glands, hyperplastic through stimulation from the pituitary gland, had become independent of the 'master gland' and that at about this time a primary hyperpituitarism had developed into a hypercorticalism. The latter would have been secondary and dependent on the pituitary stimulation at first.

Periadrenal air insufflation. In view of the possible sequence of events just described, it would be of interest to know at what time in the course of this patient's disease the adrenals had become markedly hyperplastic. Unfortunately, no definite answer to this question is available. Periadrenal air insufflation was performed twice at New Haven Hospital, in 1936 and 1938. No obvious unilateral enlargement of the adrenal glands was found, and on account of the patient's adiposity no definite estimate of the size of the adrenals was possible.[3] In 1940 periadrenal air insufflation was performed at Jefferson Hospital. Again the absence of adrenal tumors was stated, but both adrenals were definitely enlarged.

Androgen excretion. The androgen excretion in this case was high and dropped to normal values under treatment with diethylstilbestrol. At first (1940–1941) this decrease in androgen excretion coincided with considerable clinical improvement. However, there was a recurrence of symptoms in February, 1941, as described in our previous communication (2). Urinary androgen assay on May 26, 1941, showed 12 I.U. per 24 hours. No further assays were performed during the next months until her return to the hospital in January, 1942. At this time (January, 1942) the 17-ketosteroid excretion was 31 mg. per 24 hours. No bioassay of androgens was performed at that time. Although no previous determinations of 17-ketosteroids had been made, the value obtained is undoubtedly high. To our knowledge no extensive comparative study of androgen bioassay and 17-ketosteroid determination has been published. This 17-ketosteroid value cannot, therefore, be compared with the previous bioassays for androgenic material.

There remains, however, the fact that the androgen excretion decreased during treatment with diethylstilbestrol. The possibility that a decrease of androgen excretion was purely coincidental and not related to the treatment seems improbable, particularly because a decrease in androgen excretion under treatment with diethylstilbestrol was observed by us in a second case (2).

No explanation can be given for the fact that ultimately the diabetes and the osteoporosis progressed in spite of continued treatment with

[3] We are greatly indebted to Dr. K. W. Thompson of Yale University School of Medicine for this information on his findings.

diethylstilbestrol, while at the same time the androgen excretion remained at a low level. It might be possible that stilbestrol was more potent in its suppression of androgen excretion than on excretion of other cortical steroids. This explanation would assume an at least temporary dissociation of production and excretion of various cortical steroids. Seventeen-ketosteroid excretion was high at a very late stage of the disease, but unfortunately we have no bioassay of androgens of this time. It might be mentioned that at autopsy only small amounts of fuchsinophile 'androgenic' cells (3, 13) were found in the adrenals. The staining reaction did not differ from that in some of the normal control specimens and was very much less extensive than was found by Broster and Vines (13) and others. The low androgen excretion during the last year of the patient's life would be in keeping with the histologic findings. However, the significance and specificity of the staining reaction is still open to much doubt and we therefore cannot attach importance to it.

Pregnandiol excretion. As mentioned in our previous report, this patient excreted 8 mg. of pregnandiol per 24 hours in September, 1940, despite amenorrhea. Under treatment with diethylstilbestrol the excretion dropped to 6 mg. in January, 1941 (2). Under continued treatment with diethylstilbestrol the excretion decreased further to 3 mg. (June, 1941) and 2 mg. (January, 1942). It will be noted that the decrease of pregnandiol excretion continued to quite low values at a time of recurrence of symptoms.

Excretion of pregnandiol in absence of a corpus luteum is considered to be due to progesterone production by the adrenal cortex. Excretion of large amounts of pregnandiol in cases of virilism has been reported (14, 15, 16). Talbot, *et al.* (17), found the excretion of pregnandiol increased in some cases of adrenal cortical hyperplasia and normal in others.

Demineralization and calcium metabolism. Osteoporosis was not an early event in the course of the disease in this patient. None was found from 1934 to 1937. Some demineralization of the spine was first noted when the patient was in New Haven Hospital in 1938, and again when she was in Jefferson Hospital in 1939. It progressed very rapidly during the patient's last year of life. The progression of the demineralization was most marked at a time when the excretion of androgenic hormone and of pregnandiol was decreasing. Whether the treatment with diethylstilbestrol during this time failed to arrest the demineralization or actually increased it cannot be decided.

In January, 1942, a calcium balance study was performed using the dietary regime of Bauer and Aub (18). At this time demineralization was far progressed and had led to collapse of several vertebrae. The excretion of 500.7 mg. of calcium in the urine during a 3-day period, as against an intake of 330 mg., indicated a negative calcium balance. Similar observations in patients with Cushing's syndrome have been published (19, 20). The serum calcium level at this time was 10.6 mg. per 100 cc. The phosphorus was 3.3 mg. per 100 cc. and phosphatase 21.7 Bodansky units. While Ca and P values were normal even at this period, the high phosphatase values indicate a reparatory tendency of the organism. Phosphatase levels had been determined in this patient in November, 1939 (3 Bodansky U), and in October, 1940 (8.8 Bodansky U). Some demineralization had been observed at the time these values were established. Only when the demineralization had progressed to extreme degrees was the serum phosphatase significantly elevated.

Nitrogen balance. Albright, *et al.*, have suggested that in Cushing's syndrome a number of symptoms, including the osteoporosis, is due to disturbed protein metabolism. A slightly negative nitrogen balance was found and considered to be due to increased gluconeogenesis (19). In our patient a nitrogen balance study was performed in January, 1942. At this time the osteoporosis was extreme, the calcium balance negative and the diabetes marked. She had been treated with diethylstilbestrol for about a year and a half. In a 3-day observation period the N intake was 31.8 gm., the urinary output, 17.9 gm. The balance study is incomplete inasmuch as the N loss through the feces was not determined, but the values obtained do not seem to indicate a negative nitrogen balance.

Albright, *et al.* (19), have described a beneficial effect of treatment with testosterone

propionate in cases of Cushing's syndrome. The osteoporosis was improved and the negative calcium and nitrogen balance reverted to normal. Our patient received testosterone propionate for 2 weeks just prior to her death. At that time she had developed multiple purulent skin lesions, complicated by erysipelas. The failure to respond to testosterone under these conditions was to be expected.

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Contribution to the Treatment of Essential Pruritus and Kraurosis Vulvae

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THE HORMONAL treatment of essential pruritus, kraurosis and leukoplakia of the vulva gives a more favorable prospect for alleviating the symptoms and curing the conditions than any known form of therapy. Roentgen-ray therapy in a series of 22 patients with such degenerative disorders resulted in cure in 27 per cent and temporary relief in 23 per cent of the patients, the remaining 50 per cent being refractory to treatment. Estrogenic therapy in a group of 38 patients suffering from pruritus vulvae resulted in cure in 66 per cent of the cases (4).

The local use of an ointment containing estrogen was instituted because it appeared that, when injected, only a fraction of the follicular hormone is utilized by the affected vulval tissues and the prolonged parenteral treatment and high dosage was unnecessary. This mode of topical medication was started independent of Zondek's (10) studies along the same lines and at about the same time. Ten patients of our series were treated by local inunction only. This resulted in complete cure in 6 cases and proved the effectiveness of directly applying the hormone to the affected tissues. Later, however, therapy involving the simultaneous local and parenteral administration of the estrogenic substance was instituted according to the following scheme. Four gm. of a salve containing estrogen equivalent in activity to 4000 I.U. of estrone was applied to the vulva by daily inunction for the first week. This was supplemented by injections of estradiol benzoate, 30,000 international benzoate units each, given every other day. During the second week the parenteral dosage was continued whereas the ointment (4000 I.U.) was employed on the days when injections were not given. During the third week local medication was applied 2 to 3 times, according to the severity of the condition, and the parenteral dosage was decreased to 20,000 I.B.U. given twice each week. During the fourth and fifth weeks there were 1 to 2 local treatments per week supplemented by 2 injections of 10,000 I.B.U. of estradiol benzoate each. Beginning the sixth week, only percutaneous therapy was employed; 4000 I.U. were applied every 3 to 6 days. The whole course of therapy was continued over a total of 8 to 12 weeks. This, however, was not regarded as an inflexible regimen, and the dosage largely depended upon the severity and responsiveness of the case. Later, other reports of gratifying results with local administration of estrogenic ointment in degenerative genital disorders of this type appeared (1, 2, 3, 5–9). Reifferscheid (7), following our procedure in a series of pruritus cases, produced complete relief from symptoms in about 71 per cent of the patients.

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Present Material, Therapy Employed and Results

The present series of 68 cases of degenerative disorders of the outer genitalia consisted of 54 of pruritus vulvae, 6 of primary kraurosis and 8 of leukoplakic vulvitis and secondary kraurosis. There was no leukorrhea in these patients and there were no clinical manifestations of vitamin deficiency. The urine in all cases contained no sugar and the blood-sugar level was within normal range. A routine search for monilia and trichomonas vaginalis ruled out these parasites as causative factors of the pruritus.

As can be seen in table 1, two-thirds of the patients were women in the menopause. Among the women in the twenties and thirties, there were 2 patients with primary kraurosis and 3 with leukoplakia. Careful examination had

shown them to be in perfect health with apparently normal ovarian function. There was an unusual case of a 6-year-old girl with leukoplakia vulvae and subsequent kraurotic changes.

The therapy employed generally followed the same pattern as that outlined in connection with the previous series, but more emphasis was placed upon local treatment. Thus, with ment was applied morning and night on days when injections were not given. Eight weeks after the initiation of local therapy, the parenteral dosage was decreased to 10,000 R.U. a week, the topical medication being continued at the same dosage. The whole course of therapy required 12 weeks. By decreasing the dosage of the parenterally administered hormone, as compared with our previous scheme,

TABLE 1. RESULTS OF ESTROGENIC THERAPY IN VULVAL DISORDERS

Age Group	Diagnosis and Result of Therapy					
	No. of patients	Pruritus vulvae	No. of patients	Primary kraurosis	No. of patients	Leukoplakic vulvitis and secondary kraurosis
Prepuberal	0		0		1	1 cured
Reproductive age Apparently normal ovarian function	9	3 cured 2 improved 4 refractory	2	2 refractory	3	3 refractory
Hypo-ovarian function	7	4 cured 3 improved	0		0	
Menopausal	38	24 cured 4 improved 10 refractory	4	2 cured 1 improved 1 refractory	4	2 cured 2 improved

variations as necessitated by both the clinical picture presented by the patient and her response to therapy, the following scheme was applied. During the first 1 to 3 weeks local treatment only was given. This consisted of vulval inunction with a salve, each dose representing the equivalent of 2000 and 5000 I.U. of estrone,[1] depending on the severity of the condition the lower or higher concentration was used. The ointment was administered twice a day. The skin was thoroughly cleansed prior to treatment. A cotton pad soaked with warm water was applied to the area for 5 minutes in order to facilitate absorption. The ointment was rubbed gently into the affected area until it disappeared. In the second, third or fourth week, particularly in those instances exhibiting hypoövarianism aside from the vulval changes, parenteral therapy was instituted consisting of two injections of 10,000 R.U. of estradiol benzoate[1] per week. The ointside effects of vaginal bleeding and discharge were mostly eliminated in the present series. However, parenteral hormone administration was not completely discontinued because it favorably influences the general condition of the patient and the systemic manifestations of the menopause.

Table 1 shows the results of the present scheme of treatment, which are slightly less favorable in the pruritus group of this series (57 per cent cured) than in our first series (4). This may be attributed to the fact that this series included patients with only the most severe conditions which had not responded to any kind of therapy previously, including roentgen-ray. Of a total of 54 cases, 74 per cent were permanently cured or markedly improved.

The respective groups exhibiting primary kraurosis and leukoplakic vulvitis with secondary kraurosis are small, but of a total of 14 patients in these two groups 8 were cured or markedly improved. Thus, by employing the combined local and parenteral hormone therapy more than 50 per cent of our patients

[1] We are indebted to Dr. L. Pirk of Roche-Organon, Inc., Nutley, N. J., for generous supplies of an ointment containing estrone (Menformon Dosules) and the estradiol benzoate ampuls (Dimenformon Benzoate).

suffering from these degenerative disorders were benefited.

A patient was classified as cured only if a follow-up study over a period of 12 months showed her to have remained symptom-free.

CASE HISTORIES

Patient F.B., 63 years of age. Menarche at 12.5 years. Menstruation without cramps lasting 4 to 5 days, had occurred regularly every 4 weeks with a moderate amount of bleeding. There were 2 normal births and 3 induced abortions. The menopause occurred at the age of 49. Since that time there has been hypertension. The chief complaint was excruciating itching of the genitalia which prevented sleeping. Pruritus vulvae which began at the age of 60 had gradually progressed to the perineum and then to the anal region. Various local treatments including silver nitrate and a novocaine-menthol salve brought only temporary relief. Finally roentgen-ray treatment to the vulva was used resulting in some improvement for 3 months. However the pruritus recurred even more intensively.

The patient was moderately well nourished and gave the impression of being nervous and irritable. The blood pressure was 180/195 mm. Hg. Urinalysis was negative for albumin and sugar. The blood sugar was 120 mg. per cent. The heart showed enlargement of the left ventricle and accentuation of the second sound over the aortic area. There were varicose veins of the lower extremities. In the region of the genitals there were numerous scratch marks and shallow erosions on labia and in the region of the clitoris; there was partial lichenification of skin on labia majora and perineally. There was no discharge; no trichomonas vaginalis could be found. The pelvic examination showed nothing noteworthy. Rectoscopy revealed atrophic proctitis in the region of the sphincter. A diagnosis of essential pruritus vulvae was made.

An estrogenic ointment, containing 5000 I.U./gm. (estrone) was prescribed for use twice a day, and was to be rubbed into the vulval tissues. The itching increased after the second application of the estrogenic salve; however, on the fourth day the pruritus diminished, disappearing only from the anal region after one week of treatment. In the second week injections of estradiol benzoate, 10,000 R.U., were instituted. This dose was given twice a week and the salve was applied the days when there were no injections. At the end of the third week the pruritus disappeared completely. The patient slept well and expressed a sense of well being. From the fourth week, 10,000 R.U. of estradiol benzoate was injected once a week, local therapy being continued daily at the same dosage level as first prescribed except when parenteral medication was given. After completion of the 12-week period of treatment the patient remained free of complaints for one year, the period over which she was observed.

Patient J.K., 69 years of age. Menarche at 14.5 years. Menstruation lasting 6 days had occurred every 4 weeks, unaccompanied by menstrual molimina. There were 4 normal births and 2 abortions. Menopause was at the age of 49.

The chief complaints were genital itching, nervousness and insomnia. She had been suffering from the pruritus for 1.5 years and during this period 'local treatment,' the nature of which she did not specify was given with only temporary relief. Roentgen-ray therapy was used without beneficial effect.

The patient was undernourished. The blood pressure was 165/90 mm. Hg; the urine negative for albumin and sugar; the blood sugar, 110 mg. per cent. A general physical examination showed nothing remarkable. Pelvic examination revealed an atrophic uterus, cervix flush with vaginal vaults. On both labia, accentuated in the region of the clitoris, there were patches of whitish opaque and partly fissured lesions. There were atrophic changes and scratch marks in the surrounding areas. Vulval biopsy showed collagen degeneration of the subcutaneous tissue; the skin was devoid of pigment and there was atrophy of the papillary body. There was hyperkeratosis and parakeratosis. A diagnosis of kraurosis vulvae was made.

Inunction with an estrogenic salve containing 5000 I.U./gm. (estrone) was prescribed for use twice a day. After 8 days of local treatment there was complete relief of pruritus during the day. At night, however, severe itching continued, rendering the patient sleepless. In the second week injection of 10,000 R.U. of estradiol benzoate was instituted twice a week. The topical medication was employed on those days when no injections were given. Combined local and parenteral treatment brought about a better blood supply to the atrophic regions of the genitalia. In the eighth week the parenteral dosage was decreased to 10,000 R.U. a week, inunction being continued twice a day. The atrophic foci had gradually become much smaller and in the eighth week of treatment the pruritus disappeared completely. The patient felt very well and treatment was discontinued after 12 weeks. She remained free of symptoms for one year, after which time she was not seen.

Patient B.H., 61 years of age. Menarche was at 14 years. Menstruation accompanied by cramps and backaches, and lasting 2 to 3 days had occurred every 3 weeks. Nulligravida. Menopause was at the age of 45. Syphilis had been contracted in her twenties and had been treated.

The chief complain was of severe itching of the genitalia, from which the patient had been suffering for the last year. Various kinds of treatment were given without success, including roentgen-ray therapy.

The patient was a moderately well nourished person with a blood pressure of 140/85 mm. Hg. The urinalysis was negative for albumin and sugar the blood sugar, 95 mg. per cent. A general physical examination revealed nothing of significance. The patient gave the impression of being very nervous. There

were several leukoplakic foci and fissures in the region of the vulva, as well as shallow ulcerations at the posterior commissure. There were no local lesions in the vagina; the uterus was atrophic. A diagnosis of leukoplakia vulvae was made.

Therapy consisted of local application of a hormone ointment containing 5000 I.U. of estrone to be used twice a day. Healing of the fissures and ulcerations occurred in the second week and was accompanied by diminution of the pruritus. Ten days after institution of therapy parenteral treatment was started using diphtheria. At the age of 4 a severe pruritus vulvae developed which was relieved to some extent by symptomatic treatment; however, as the mother told us, there were recurrences at certain intervals.

The patient gave the appearance of a healthy, normally developed child. Tuberculosis and syphilis were absent. A complete physical examination[2] revealed nothing remarkable. There were no skin lesions other than those at the vulva. Repeated urinalysis did not show any deviation from normal. No worms or their eggs could be detected in the stool. The hemo-

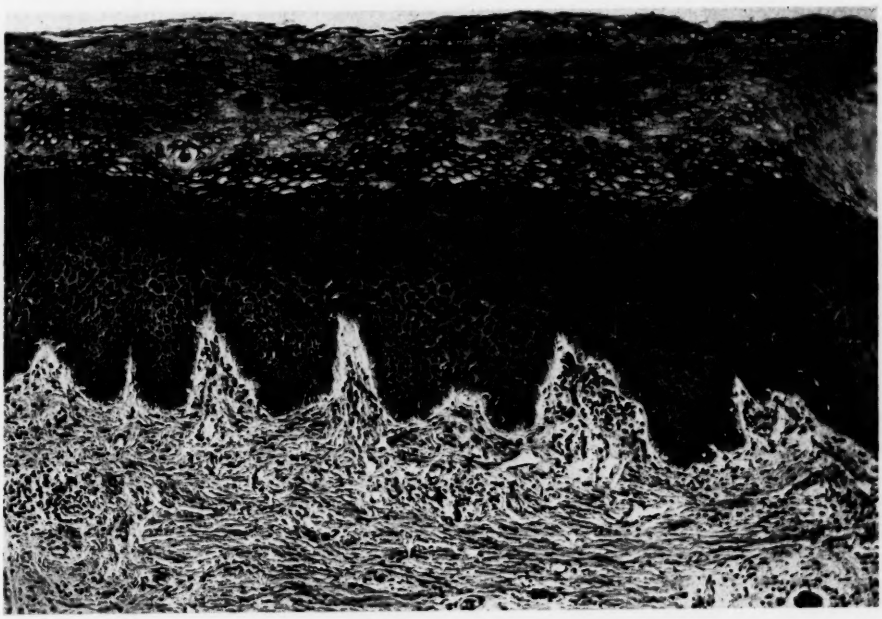

Fig. 1. Biopsy of vulval tissue of 6-year-old patient, *G. H.*, revealing leukoplakic vulvitis.

10,000 R.U. of estradiol benzoate twice a week. The local inunction was maintained on days between injections. The blood supply of the skin improved noticeably during the following weeks. The opaque whitish foci became smaller and the fissures disappeared completely. Itching occurred only at night but it was less frequent and less intense. Beginning with the eighth week the parenteral dosage was decreased to one injection of 10,000 R.U. of estradiol benzoate a week. After completion of the 12-week course of therapy there was no pruritus, no ulcerations and no fissures, with only minute opaque foci. The symptoms did not recur during the follow-up study of 1 year.

The child who was observed and successfully treated is the youngest patient with leukoplakia and kraurosis vulvae on record so the complete case history is given.

Patient, G.H., 6 years of age had had measles and globin was 78 per cent and the color index 0.88. A blood count showed 4,490,000 erythrocytes per cu. mm. and 8,400 leukocytes per cu. mm. The blood platelets were normal in number. There was a moderate anisocytosis and a slight anisochromia. The white blood picture did not show a shift to the left. However, there were 2 per cent of eosinophilic cells. The large monocytes were of normal structure; there was a lymphocytosis of 48.5 per cent.

Around the clitoris and the region of the labia minora there appeared opaque, whitish, circumscribed areas which were confluent, giving the clinical picture of leukoplakia vulvae. Biopsy revealed a marked hyperkeratosis and parakeratosis associated with pronounced edema of the subepithelial layers. The papillae were flattened and atrophic. In the subepithelial

[2] We are indebted to Dr. R. Wagner of Boston, Massachusetts, for pediatric examination of this patient.

layer there was an extensive infiltration of lymphocytes and plasma cells; these were most marked within the papillary layer. The histologic picture of the excised specimen was typical of leukoplakia vulvae (fig. 1).[3]

Later there appeared small circumscribed atrophic areas above the clitoris and within the labia minora and tiny folds became apparent; in the subsequent course of the disease a kraurosis vulvae developed in addition to the leukoplakia. There was a fragmentation and decrease of the elastic tissue fibers and a marked atrophy of the papillae.

The child was subjected to the same type of therapy as used in the previous cases; however, the dosages of estrogenic hormone employed were much smaller than those administered to the adult patients. In the first week inunctions containing 2000 I.U. of estrone each, twice a day were applied to the vulva. In the second week 2000 R.U. of estradiol benzoate were injected intramuscularly every 3 or 4 days and on the days she did not receive injections ointment application to the vulva was continued each morning and evening using a total of 4000 I.U. for the day. After 3 weeks the injections were discontinued, only local treatment twice a day being administered using 2000 I.U. of estrone each time. Four weeks after institution of therapy there was marked improvement in the condition. The pruritus had subsided and the mucous membranes of the external genitalia were well supplied with blood. The whitish areas diminished in size but the kraurosis at this time did not show signs of improvement. The inunction was continued for a total of 12 weeks and a remarkable improvement of all the affected tissues was achieved. Considering the age of the patient and the excellent clinical response, it was thought inadvisable to take a post-medication biopsy specimen for histologic study.

DISCUSSION

The majority of cases of essential pruritus vulvae, kraurosis vulvae and leukoplakic vulvitis occur after the menopause. In view of the fact that a high percentage of cures in such cases is effected by estrogenic therapy, it is apparent that declining ovarian activity is an important etiologic factor. However, there are other causative factors in the pathogenesis of these disorders such as predisposition of the vulval tissues, familial factors and probably other factors unknown to us. That constitutional factors play a rôle is manifested by the fact that only a small fraction of menopausal women suffer from pruritus or kraurosis vulvae; furthermore, such disorders are found in women of the reproductive age group, even in girls shortly after puberty, and in our series, in the case of the 6-year-old child. Among the familial factors in cases of essential pruritus vulvae were found disposition toward functional ovarian disturbances, myomata, obesity, metabolic disorders and diabetes, as well as functional disturbances of other glands.

It is significant that of the cases of kraurosis and leukoplakia which were refractory to hormone treatment, the majority belonged to the group of young women with apparently normal ovarian function. Failure of therapy in all likelihood indicates that in these instances elements other than decreased ovarian activity were etiologic factors in the disorders. The importance of vitamin-A deficiency has been stressed. Also it is noteworthy that over 13 per cent of our patients with kraurosis and leukoplakia, the etiology of which still remains obscure, had a previous syphilitic infection.

Of the 9 pruritus cases in patients with apparently normal ovarian function, 3 were cured and 2 were markedly improved by treatment. It may be assumed, although there is no conclusive evidence, that these 5 had an estrogenic deficiency as an underlying cause of the disease. However, the estrogen in these instances might have acted pharmacodynamically.

SUMMARY AND CONCLUSIONS

A series of 68 cases of essential pruritus vulvae, kraurosis vulvae and leukoplakic vulvitis with secondary kraurosis is presented. A treatment scheme, employed in all instances and consisting of local application of estrone ointment and parenteral administration of estradiol benzoate, is outlined.

Careful evaluation of results shows that in the group of 54 pruritus cases, 74 per cent were cured or markedly improved. There were 6 cases with primary kraurosis and 8 with leukoplakic vulvitis and secondary kraurosis. Of these 14 patients, 8 were either cured or markedly improved. Among the cured was the unique case of a 6-year-old girl with leukoplakia vulvae and subsequent kraurotic changes. The case history of this patient is given in detail. Also, 3 other case histories are presented.

[3] We wish to express our appreciation to Dr. Paul Klemperer, Clinical Professor of Pathology at Columbia University and Attending Pathologist at Mt. Sinai Hospital, who inspected this specimen and confirmed our diagnosis.

The importance of ovarian failure in the pathogenesis of these disorders is stressed. However, it is suggested that causative factors other than hypoövarianism may play an important part. It is in these instances particularly that estrogenic treatment might fall short of expectations.

Since no other method of therapy affords as large a percentage of cures in cases of this type as does the use of combined local and parenteral administration of estrogenic hormone, cases of essential pruritus vulvae, kraurosis vulvae and leukoplakic vulvitis should be given the benefit of this type of treatment.

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Effect of Estradiol and Diethylstilbestrol upon the Atrophic Human Buccal Mucosa with a Preliminary Report on the Use of Estrogens in the Management of Senile Gingivitis

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IT HAD BEEN noted by one of us (A. R. A) that many climacteric patients complain of a dryness or burning sensation in the mouth. Following therapy with estradiol or diethylstilbestrol, these symptoms would disappear. An investigation of the literature disclosed that the reports on this subject were few. Nathanson and Weisberger (1) clearly described the associated gross lesions before and after treatment with estradiol, but they made no report of a microscopic study. In the castrated female monkey, Ziskin described stimulation and growth of the gingival mucosa following treatment with estrone and estradiol (2). In the human female, on the basis of a single biopsy specimen from each of 3 patients receiving estradiol, Ziskin concluded that estrogen stimulates growth and keratinization of the gingival mucosa (3).

These studies were made in view of the incomplete reports on mucosal response to estrogens in human beings. Estradiol and diethylstilbestrol were used in order to determine if there was any difference in their action. The substances used were estradiol dipropionate,[2] diethylstilbestrol[3] and diethylstilbestrol monomethylether.[4] The latter two were administered orally. The estradiol dipropionate and monomethylether of diethylstilbestrol were injected either intramuscularly or locally under the buccal mucosa.

Twenty-five climacteric patients, observed for 3 to 18 months, form the basis of this report. In 17 patients, the menopause had been induced surgically. Gross oral manifestations, subjective symptoms and the histologic structure of the buccal mucosa before, during and after treatment were correlated. Biopsies were made under local anesthesia from the region of the buccal mucosa opposite the first and second molars. Vaginal biopsies were made, if there was an atrophic condition as determined either grossly or by vaginal smear. Since these studies will be reported in detail elsewhere (4), the results obtained will be briefly presented for their endocrine interest.

Correlation of Visual Oral Manifestations with Subjective Symptoms and Biopsies

Observations before treatment. In general, the longer the interval of time since the menopause, whether surgical or spontaneous, the

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[1] Now with the Department of Obstetrics and Gynecology, George Washington University School of Medicine, Washington, D. C.

[2] The estradiol dipropionate (Di-Ovocylin) was supplied by Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, N. J.

[3] The diethylstilbestrol (Estrobene) was supplied by Mr. A. A. Ebby of Ayerst, McKenna and Harrison, Inc., Rouses Point, N. Y.

[4] The diethylstilbestrol monomethylether (Monomestrol) was supplied by Dr. W. Salmon and Dr. F. Schmelkes of Wallace and Tiernan, Inc., Belleville, N. J.

more frequent were the varying degrees of atrophy and hyperkeratosis of the buccal mucosa. Within 6 months after the cessation of menses, the oral mucosa tended to be a normal rosy pink in color, moist and smooth. Biopsies revealed a normal histologic structure. In patients in whom the menopause had occurred 6 months to 2 or more years before, the mucosa usually was pale, grayish pink and anemic in appearance. At about this time, complaints were made of a dry or burning sensation in the mouth. Biopsies usually disclosed beginning atrophy of the prickle cell layer with an occasional early keratosis of the corneal layer.

In those cases in which the menopause had occurred more than 2 years before, milky, whitish or gray areas were noted more frequently, occurring either diffusely or in patches. At the same time the gingivae were observed to bleed easily, especially when the teeth were brushed or on slight pressure. Burning or dryness of the mouth was complained of more frequently. Biopsies of the buccal mucosa disclosed that atrophy of the prickle cell layer in these patients was more advanced while keratinization of the corneal layer was more marked. Macroscopically there were areas in which there was a milky white adherent film. Tissue from these regions showed a true leukoplakia on microscopic examination.

In both the early stages of simple atrophy and the later stages of keratosis and leukoplakia the histologic resemblance to similar lesions of the vulvovaginal tract was striking. No definite correlation, however, of the condition of the vulvo-vaginal mucosa and the buccal mucosa could be found.

Observations after treatment. With the exception of the leukoplakic areas in some patients, the mucosa became a normal bright pink in color following treatment. Several reported an increased salivation. The sensation of burning or dryness and the tendency for the gingivae to bleed disappeared early. Histologically, the following changes were observed. There was a marked increase in the vascularity of the submucosal area, especially evident in the rete papillae. The basal layer which showed the most dramatic changes was greatly increased in thickness. The cells were larger. Occasional mitoses were found. The corneal layer became thicker and showed more keratinization. On the other hand, the leukoplakic areas showed no increase in keratosis and in some instances there was a decrease.

It was found that injection of estradiol dipropionate locally into the buccal mucosa was the most effective and efficient method of administration. Concomitantly the hot flushes were also relieved. Both diethylstilbestrol and estradiol had the same effect upon the atrophic buccal and vaginal mucosa.

DISCUSSION

From the results obtained in this study, it is apparent that the natural steroid, estradiol, and the synthetic nonsteroid, diethylstilbestrol, are equally efficacious in restoring the atrophic buccal mucous membrane to a normal condition. The gross observations in these cases are in agreement with those of Nathanson and Weisberger except that they noted grossly a disappearance of the leukoplakic areas, while, as a rule, we did not. The microscopic observations confirm those of Ziskin in the monkey, indicating that the cellular reactions are similar in the mucosa of the human female and the monkey.

In its broader aspects, these studies supply additional evidence that the so-called 'sex' hormones are chemical substances capable of materially influencing many diversified phases of the body economy and that these effects are independent of the hormone action upon the secondary sexual characteristics.

Clinical implications. From a clinical point of view, the observation that the gingivae no longer bled easily after treatment with estradiol or diethylstilbestrol is significant. This fact has now been utilized by one of us (M. J. R.) in the management of senile or atrophic gingivitis. In this condition, the gingivae develop atrophic changes as described herein. In addition, they are characterized by a free bleeding which occurs under any pressure or tooth-brushing. In a selected number of these cases, in both men and women, estrogenic therapy was administered. Since the experimental evidence had shown that local therapy was the most effective, this has been used as

the route of administration. Two methods of local application were tried. In some patients, diethylstilbestrol ointment was massaged into the gum tissues with rubber applicators. In other cases, estradiol dipropionate was injected directly into the muco-buccal fold adjacent to the area to be treated. Of the two methods, the injection technique proved more effective. The inunction of diethylstilbestrol ointment, however, proved to be good adjuvant therapy after the initial result was obtained by injection. Bleeding from the gums ceased in all treated cases. These studies will be reported in detail elsewhere.

SUMMARY AND CONCLUSIONS

Gross oral manifestations, subjective symptoms and histologic structure of the buccal mucosa were correlated before, during and after treatment with diethylstilbestrol, diethylstilbestrol monomethylether and estradiol dipropionate in 25 climacteric patients.

Following the menopause a sequence of atrophic changes in the buccal mucosa may develop in the course of several months or years. Grossly, the normal bright pink color may become pale and anemic. Later, grayish white areas may appear diffusely, in patches or in streaks. Finally, areas containing a whitish adherent film may be observed. Associated with these atrophic changes, a sensation of dryness or burning may be noted by the patient. Histologically, the stratum germinativum atrophies; the corneal layer, with the lapse of time and under the influence of irritation which produces an abnormal growth stimulus, may develop leukoplakic areas.

The administration of estradiol or diethylstilbestrol provides a normal metabolic stimulus to the buccal mucous membrane. The latter develops a normal growth pattern. The prickle cell layer hypertrophies, while the corneal layer becomes keratinized. The leukoplakic areas may tend to shrink. Grossly, the oral mucosa resumes its normal moist bright pink color.

Clinically, the sensation of burning and dryness disappears while the gingival bleeding is controlled. These findings have been utilized to good advantage in the management of senile atrophic gingivitis. The local injection of estradiol dipropionate beneath the muco-buccal fold proved to be the most efficacious method of administration.

The authors are indebted to Dr. H. C. Falk, Director, and Dr. P. M. Murray, of the Department of Gynecology and Dr. Louis H. Fairclough, Director of the Department of Oral Surgery, for permission to carry on this work.

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Nutritional Deficiency in the Etiology of Menorrhagia, Metrorrhagia, Cystic Mastitis and Premenstrual Tension; Treatment with Vitamin B Complex[1]

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EVIDENCE relating the occurrence of certain forms of pathologic uterine bleeding, and of premenstrual tension, chronic cystic mastitis and other disturbances to an excess of estrogen has been accumulating for some years. This evidence was provided originally by the fundamental work of Frank and his collaborators (1–3). Other data have been summarized by several authors in recent authoritative symposia (4, 5). In addition to the investigations there described, Ehrlich (6) has observed in endometrium from patients bleeding abnormally, thrombotic phenomena similar to those illustrated by Zuckerman (7) in the endometrium of castrated monkeys treated with estrogen.

By utilizing an ingenious method devised by G. R. Biskind (8, 9), in recent studies on the steroid-inactivating mechanism of the liver in rats, it has been shown that the liver loses its ability to inactivate estrogen in vitamin-B-complex deficiency (10–12). Addition of brewer's yeast to the diet was found to restore the inactivating mechanism (12). The amount of estrogen inactivated by the liver could be controlled at will, by withholding the vitamin B complex or by restoring it to the diet. At the same time it was found that the inactivation of androgen was not significantly impaired in vitamin-B-complex deficiency (13), thus leading to a serious alteration of the estrogen-androgen equilibrium. The phenomena described occurred in the absence of detectable gross or histologic lesions in the liver. Significant diminution in the ability of the liver to inactivate estrogen occurred in some animals with only a slight or moderate degree of vitamin B deprivation. In previous papers (11–13), we have pointed out some of the possible consequences of the rise in body estrogen and alteration of the estrogen-androgen equilibrium which occur in vitamin-B-complex deficiency.

On the basis of these investigations, it seemed worth while to determine whether there was a clinical correlation between nutritional deficiency and the occurrence of syndromes related to an excess of estrogen. Accordingly, a study was undertaken to determine whether patients with signs and symptoms of nutritional deficiency had menorrhagia, metrorrhagia, painful breasts, premenstrual tension or a combination of these. Conversely every patient presenting one or more of these conditions was investigated as to her nutritional status. As a definite correlation was found, treatment of these endocrine conditions with vitamin B complex was instituted.

CASE REPORTS

Case 1. R.G., age 46, had had menorrhagia for 5 years. During this time two periods had been normal. These had occurred following a curettage 8 months before she was referred to the Beth Israel Endocrine Clinic. Bleeding had by that time become so profuse that radiation therapy was advised by the family physician. For more than 4 years before the curettage bleeding had been irregular occurring, at first, every 5 or 6 weeks, with an occasional longer interval, and

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later at intervals as short as two weeks. Following the curettage the periods had been more regular, occurring every 25 to 30 days, but except for the two normal periods already mentioned, bleeding lasted 9 days. There was no history of tenderness of the breasts.

The height was 61 inches, weight 151 pounds. Gynecologic examination revealed no detectable abnormality in uterus or adnexae. There was a definite but not severe atrophic glossitis when she was first seen; the diet had been deficient for many years.

The patient was treated chiefly with a fortified preparation of rice bran extract[2] and, for part of the time, with a preparation derived from yeast.[3] The first subsequent period, after 3 weeks of therapy, appeared a week before it was expected; the flow was scant and lasted 3 days. By this time the tongue had completely healed. The next two periods occurred at the expected times; one lasted 6 days, the next, 3 days. Bleeding was much less profuse than it had been and the patient remarked that she felt much better during the periods than formerly.

Case 2. M.G., age 42, had a severe atrophic glossitis, cheilosis[4] and ulcerations inside the lower lip. She had had menorrhagia for about 4 years; the menstrual periods occurred every 28 to 30 days and lasted from 4 to 10 days. Associated with the menstrual disorder and the oral signs of nutritional deficiency, there had been over a period of years a variety of physical and mental symptoms. She was depressed, apprehensive, exceedingly irritable and was concerned because she was getting increasingly short-tempered with her children. She had aches and pains in arms and shoulders and suffered from many headaches. These symptoms all became worse just before and during each period. They were so severe that she had had repeated psychotherapy, without apparent benefit. The diet had been deficient over a long period of time; whole grains and fresh fruits and vegetables especially were lacking. Calorically, however, the diet was more than adequate, as she weighed 161 lb. and was but 64 inches in height. She had had a thyroidectomy 11 years before; the basal metabolic rate currently varied between minus 21 and minus 12. The thyroid was diffusely enlarged and prominent. Repeated attempts to administer thyroid had resulted only in increasing the patient's nervousness and irritability and it had had to be discontinued. Gynecologic examination revealed no abnormalities in the uterus or adnexae.

For 5 months, *M.G.* received vitamin B complex orally, mainly a preparation derived from yeast,[3] 3 times a day with meals. After 5 weeks this was supplemented with thiamin 9 mg., riboflavin 6 mg. and nicotinamide 75 mg. per day in divided doses.

After two weeks of therapy, a period occurred which lasted 4 days; spotting occurred for two days, the flow was fairly profuse for the next two days. When the patient was seen at the end of this period the glossitis was much improved, the cheilosis unaffected. The next period occurred 30 days later, lasted 3 days and the flow was normal in amount. By this time the tongue had almost completely healed and the ulcers in the mouth had disappeared, but the cheilosis still showed no improvement. The subsequent 3 periods, which occurred at about 30-day intervals, lasted 2, 5 and 3 days, respectively, and were much less profuse than formerly.

After 4 months of therapy the glossitis, stomatitis and menorrhagia had improved markedly, but the cheilosis was still unaffected and the mental symptoms were only moderately improved. An intertriginous eruption on the thighs that had resisted dermatologic therapy cleared up completely. The patient was then given thiamin hydrochloride, 50 mg. intramuscularly, 3 times a week. After the second injection there was a striking improvement of the mental state; for the first time the patient was smiling and cheerful when she presented herself for treatment and except for occasional setbacks apparently due to persistent psychogenic factors, has continued so to the time of writing. Although riboflavin was administered only by mouth, when thiamin was administered parenterally, the cheilosis began to improve for the first time. The possible significance of this observation will be discussed later.

Case 3. R.B., age 30, complained of menorrhagia alternating with intervals of amenorrhea for 6 years. When the patient was first seen, the last previous period had begun 25 days before, had lasted 9 days and the flow was moderately profuse. The previous period had occurred 3 months before that and had also lasted 9 days. Only a few weeks prior to this she had been admitted to the hospital for a curettage because of severe metrorrhagia. The endometrium had been hyperplastic and edematous and had showed no secretory change. An endometrial biopsy in 1938 had shown a similar histologic picture. In 1934 this patient had had a left oöphorectomy because of a cystic ovary, and a cholecystectomy. She had been under treatment for vaginal trichomoniasis for several months.

[2] Fortified rice bran extract (Elixir Ribranex) supplied through the courtesy of the Research Laboratories of the S. M. A. Corporation, Chagrin Falls, Ohio. The usual dosage was 2 teaspoonfuls 3 or 4 times a day.

[3] This preparation (Blexin tablets) was supplied by the International Vitamin Corporation, New York through the courtesy of Mr. Milford H. Wise. The dosage employed was 6 to 8 tablets, three times a day.

[4] Cheilosis is a designation proposed by Sebrell and Butler in 1938 to describe lesions of the lips characteristic of riboflavin deficiency in human beings. The condition is described by Sebrell [Human Riboflavin Deficiency (Ariboflavinosis), in The Biological Action of the Vitamins, edited by E. A. Evans, Jr., University of Chicago Press, 1942, pp. 75–76] as follows: 'The changes . . . consisted of lesions on the lips, which began with pallor of the mucosa in the angles of the mouth. This pallor was soon followed by maceration; and within a few days superficial linear fissures, usually bilateral, appeared exactly in the angle of the mouth. These fissures showed very little inflammatory reaction, remained moist, and became covered with a superficial yellowish crust, which could be scraped off without bleeding. In some instances these linear fissures showed a tendency to extend onto the skin of the face but did not extend into the mouth. At the time the fissures were developing, the lips became abnormally red, shiny, and superficially denuded along the line of closure.' Cheilosis is considered synonymous with 'perlèche.'

This patient was overweight (159 lb., height 61 in.). The basal metabolic rate was plus 5. The thyroid was slightly and diffusely enlarged. The tongue had a striking patch of atrophic glossitis about 1.5 cm. in diameter on the right posterior portion. She had been unaware of this lesion as the tongue had not been tender or irritated. The diet had consisted chiefly of carbohydrates, no whole grains and few fruits and vegetables.

She was treated with rice bran extract[2] over a period of 3 months. Within 8 days the tongue had healed completely and the site of the previous glossitis was not detectable. The first subsequent menstrual period occurred after 7 weeks of therapy (10 weeks after the previous period); this lasted 6 days and the flow was normal in amount. The next period was 20 days later, lasted 5 days, and also was normal in amount.

Case 4. M.W., age 15, complained of vaginal bleeding for the preceding 4 months. There was a continuous intermenstrual spotting which ceased for two days before each period. The menarche had occurred at age 13 and subsequent periods were scant and irregular, occurring every 2 to 6 weeks and lasting 5 days. The only definite sign of nutritional deficiency was edema of the tongue, which showed fixed, deep indentations from contact with the teeth. The diet had long been inadequate, apparently because of rebellion against an oversolicitous mother. The thyroid was slightly and diffusely enlarged. The hemoglobin was 80 per cent. She complained of frequent attacks of abdominal discomfort and pains in the lumbar region and legs. The attacks of abdominal pain had led to an appendectomy 3 years previously, without relief of the symptom. Rectal examination showed the uterus to be definitely enlarged. There were no other abnormalities.

This patient was given a preparation derived from yeast,[3] for a period of 2.5 months. There was no intermenstrual spotting after the first subsequent period, which occurred after a 29-day interval and lasted 4 days with normal amount of flow. The succeeding period occurred 34 days later, lasted 5 days and was normal in amount. The dosage of B complex was reduced about one-half; after 11 days spotting began again. The amount of B complex was increased to the previous level and the spotting stopped after 3 days and has not again recurred. During the 2.5 months of therapy, there was a striking improvement in general physical appearance and there have been no further attacks of pain in abdomen, back or legs. The edema of the tongue likewise disappeared.

Case 5. F.H., age 39, chiefly complained of crusted lesions about the nares and upper lip of two years' duration, dating from the birth of her baby. She had a deeply-fissured tongue and puffy, bleeding gums. She had felt continuously 'exhausted' since childbirth and has had a progressively increasing feeling of 'incompetence,' and an inability to cope with life in general. Her memory for recent events had become so poor as to prove embarrassing and alarming; occurrences of the morning were already vague and distant by evening. She complained of numbness and pain in arms and hands, especially at night. The last menstrual period had been so profuse that she had been afraid she 'had a hemorrhage.' She could not remember its exact duration. She recalled that for several years she had usually passed large clots during menstruation but had not thought this abnormal. The breasts were usually tender for several days before each period; there was no palpable mastitis.

This patient was given rice bran extract,[2] 4 times a day. The first subsequent period occurring after 3 days of therapy, was quite profuse and lasted 3 days. Two and a half weeks later, there was a marked change in the appearance of the patient; she felt stronger and not so tired; her memory had improved moderately. The lesions on the upper lip and nostrils had almost completely healed and were nearly invisible; the glossitis had also improved. This case is included to show the correlation between signs of nutritional deficiency and menorrhagia.

Case 6. S.R., age 35, had had persistently painful breasts since a pregnancy 5 years previously. Associated with this was severe premenstrual tension for the last week or 10 days of each intermenstruum. She had had psoriasis for 12 years and rheumatic arthritis for 8 years (so-called arthropathic psoriasis). For several years, in addition to the painful breasts (the pain and tenderness were more marked on the left side), there had been repeated attacks of redness and swelling of the left breast associated with high fever of several days' duration. On a few occasions this had proceeded to abscess formation and required surgical drainage. Usually these attacks occurred one or two days before the onset of menstruation; a few had occurred on the last day of the period. For about 6 months she had received estrogen therapy and there had been a diminution in the severity and frequency of the attacks of mastitis; subsequently, however, while she was still receiving estrogen therapy, two severe attacks occurred during the intermenstruum. She was then advised to have a left mastectomy as the only adequate therapeutic solution. At this time she was referred to the Beth Israel Endocrine Clinic.

The menstrual history of this patient was not remarkable, the periods occurring every 26 or 27 days and lasting 3 or 4 days; the flow was normal in amount. Gynecologic examination had revealed no abnormalities in uterus or adnexae. She was 64 inches tall, and weighed 170 lb. She had two children, 11.5 and 5 years of age. There had been no other pregnancies. The basal metabolic rate was minus 4. Both breasts were tender and nodular, the left more so than the right. There was a large patch of psoriasis under each breast and similar lesions on the thighs and scalp. There was pain on movement of fingers, wrists, ankles and toes but this was not sufficient to be disabling and there was no swelling or limitation of movement in these joints at the time of examination. At this time also, there were no lesions in the mouth which suggested nutritional deficiency, although there was a history of

cheilosis at the angles of the lips before each period (a severe attack of cheilosis appeared subsequently). The diet had been very limited for several years because of restrictions imposed for the treatment of the psoriasis. She had been taking 50,000 units of vitamin A daily.

For more than 4 months *S.R.* received a preparation derived from yeast,[3] taking it 3 times a day. The tenderness and nodular quality of the breasts disappeared and the patient remained free of mammary pain and tenderness for 3.5 months. Simultaneously, the premenstrual tension subsided almost completely. The symptoms recurred and the breasts again became nodular about two weeks after the vitamin therapy was stopped (one week after the previous period). She was impressed with the necessity of continuing the vitamin therapy, which she did after a total lapse of 4 weeks. One week after the subsequent period there was another attack of redness and swelling of the left breast with a fever of 103° F. This subsided completely in two days. Following this a severe cheilosis developed involving the whole of both lips and patches of the skin about them. The dosage of vitamin B complex was then increased, another product[5] being substituted. At the same time the patient was advised to include in her diet liver, pot cheese and liberal quantities of whole grains, fruits and vegetables. Two months later there was only slight tenderness of the breasts, which were now much less nodular. There had been no premenstrual tension during this interval and no acute mastitis. Except for the lesions on the scalp, the psoriasis had improved markedly; the patches under the breasts were now much smaller. There had been no attack of arthritis since the therapy was begun, the first time since the disease had started 12 years before that she had gone through even part of the winter free of joint pain. Aside from this improvement, the patient was enthusiastic over the state of her health.

Case 7. P.W., age 35, had a nutritional deficiency secondary to chronic enteritis of 9 years' duration. There was a history of menorrhagia and cystic mastitis for the greater part of this time. The breasts had been nodular and continuously painful for several months at a time. The mastitis had been treated successfully with estrogen over a period of 6 months. The menorrhagia later gradually regressed (she had subsequently received vitamin B complex orally as treatment of the enteritis). Several years afterward, although oral B complex had been continued in the meantime, the breasts began to be painful for the latter 2 or 3 weeks of each intermenstruum. This was associated with premenstrual tension. Following treatment parenterally with a B-complex preparation derived from liver, the mammary tenderness subsided and the nervous tension diminished about 12 hours after each injection, recurring in 24 to 36 hours. Therapy with this preparation was interrupted for several months because of the development of sensitivity to the extract. During this time the mastitis and premenstrual tension became progressively worse each month. Then for 5 months, a mixture of synthetic B vitamins[6] was injected intramuscularly every second day (in each dose: thiamin 10 mg., riboflavin 5 mg., pyridoxine 5 mg., calcium pantothenate 5 mg., niacin amide 50 mg.).[7] The tenderness and nodular character of the breasts rapidly regressed and there has been no mammary pain or tenderness for more than 4 months. During the first month of this therapy there was moderate premenstrual tension, during the succeeding 4 months virtually none. Coincident with improvement in the mastitis and premenstrual tension, acne which, for several years past, had appeared during the last one or two weeks of each intermenstruum, cleared up and has not recurred.

The necessity for adequate parenteral therapy when there is interference with enteric absorption, or failure otherwise to respond to oral administration, is emphasized in this case.

In addition to the cases presented there were 22 patients with atrophic glossitis, cheilosis or stomatitis, or a combination of these, who also had a history of menorrhagia, metrorrhagia or both. Six had painful breasts, 3 had severe premenstrual tension in addition. In two other cases with signs of nutritional deficiency, 'painful breasts' was the chief complaint; the menstrual flow was normal in amount in one, scant in the other. Among the 22 patients, 6 were in the menopause at the time they were first seen and all 6 had had premenopausal menorrhagia.

Of those with marked symptoms related to excess estrogen, 6 cases in addition to those presented in detail, were treated with rice bran extract.[2] All had menorrhagia; 3 had premenstrual tension also. All responded favorably to the vitamin B complex.

DISCUSSION

It has long been known that menorrhagia and metrorrhagia may occur early in the course of cirrhosis of the liver (14, 15). Excessive uterine bleeding has been reported also in intoxication with a number of liver poisons

[5] Vitamin B complex (Betaplexin Capsules) supplied by Winthrop Chemical Co., New York City. The dosage employed in *case 6* was 3 capsules 3 times a day.

[6] Synthetic B vitamins (Betasynplex Niphanoid) supplied by the Winthrop Chemical Co. I am indebted to Dr. J. B. Rice and Mr. W. E. H. Caldwell of the Department of Medical Research of the Winthrop Chemical Company, Inc., for an experimental supply of this preparation and of the Betaplexin referred to in footnote 5.

[7] In rats a mixture of the first four of these substances has been found capable of restoring the estrogen-inactivating function of the liver impaired by deprivation of the B complex—unpublished observations.

(lead, benzene, carbon disulfide, 16–19). Cirrhosis of the liver is now known, from the work of György and Goldblatt (20–22) and of others, to result from nutritional deficiency. Sources of the vitamin B complex have been shown to protect the liver against a variety of agents (such as lead, arsenic, carbon tetrachloride, dimethylaminoazobenzene) which cause functional and morphologic damage to this organ (23–30). In addition to this evidence, Goldberger (31) has reported that menorrhagia may occur in pellagra.

Glass, Edmondson and Soll (32) have demonstrated in male patients with cirrhosis of the liver that the estrogen in the urine is increased and appears in active (uncombined) form, while the urinary androgen is somewhat reduced in amount and all of it continues to be excreted in combined form. These patients had gynecomastia, testicular atrophy, or both. Wu (33) has observed histologic changes in the prostate glands of patients with cirrhosis of the liver, indicative of stimulation by estrogen. The results we have obtained with deprivation of the vitamin B complex in rats indicate that our experimental findings and the observations of Glass and his collaborators and of Wu in hepatic cirrhosis of the human being, have a common basis.[8]

Frank (3) has reported the presence of free estrogen in considerable concentration in the urine of a puberal patient with excessive uterine bleeding. While other cases of menorrhagia and metrorrhagia investigated by him had an elevated level of urinary estrogen, the latter did not appear in significant quantity in free form. Whether the estrogen that escapes inactivation in the human liver is necessarily excreted in free (lipo-soluble) form has yet to be determined; and whether or not qualitative differences in this respect occur at various stages of, or in different kinds of, nutritional deficiency also remains to be investigated. The rôle of the kidney in this process is not yet well understood.

While this study was under way, Bean (34) published extremely interesting observations indicating that nutritional deficiency in human beings leads to an increase in body estrogen. He found that the cutaneous vascular spiders and palmar erythema formerly associated mainly with cirrhosis of the liver, occur also in nutritional deficiency and at the period in pregnancy when estrogen increases significantly. Administration of estrogen to patients with the cutaneous phenomena led to the appearance of new lesions and exacerbation of those already present; withdrawal of the estrogen caused regression of the vascular disturbances. Bean has correlated his observations with experimental studies by Reynolds and Foster (35), who showed that estrogen causes dilatation of the minute vessels in the ear of the castrated rabbit, and he has shown that the cutaneous vascular spiders are histologically similar to the spiral arteries of the endometrium observed by Bartelmez and Markee (36) and by Jones and Brewer (37). Six of the patients in the group reported here (including *cases 2* and *5*) had the cutaneous vascular spiders described by Bean.

Perera (38) has also reported the appearance of palmar erythema in nutritional deficiency; he noted regression of this lesion in two cases of hepatic cirrhosis under dietary and vitamin therapy.

Several of the patients studied in the group reported here had periods of amenorrhea alternating with menorrhagia (cf. *case 3*). Frank (3) has reported a high urinary estrogen in some cases of amenorrhea. As it is known that a hyperplastic endometrium need not necessarily bleed (especially in the absence of a suitable estrogen cycle) nutritional deficiency may also be implicated in the etiology of such cases.

In a previous publication (13) we have suggested that deficiency in the vitamin B complex may set up a vicious cycle. For not only does vitamin B deprivation impair the inactivation of estrogen in the liver, but as Ashworth and Sutton (39) have shown, estrogen may cause vitamin B deficiency. They have noted spontaneous exacerbations of signs of B deficiency during the menstrual cycle (40) and

[8] In an as yet uncompleted survey of necropsy records in collaboration with Dr. L. H. Biskind of Mount Sinai Hospital, Cleveland, Ohio, we have found evidence that there is a high level of estrogen in female patients with cirrhosis of the liver. Thus, among 16 such cases in which data were available on the pelvic organs, 6 had uterine fibroids, 6 others, endometrial polyposis, one a cervical polyp; in another case there was malignancy of the cervix; of the remaining two cases, in one there was a hydrosalpinx and in the other, paratubal cysts.

have been able to produce such exacerbations by the administration of estrogen. Among the cases I have studied, in three cyclically recurring signs of B deficiency have been observed. One patient had a severe premenstrual stomatitis associated with cystic mastitis and premenstrual tension since the birth of her child 5 years previously. The stomatitis healed promptly with large doses of vitamin B complex orally[2] and parenterally.[6] Another patient, (*case 6*) had a history of cheilosis during the last week of each intermenstruum. A third patient (*case 7*) had premenstrual acne which disappeared under combined oral and parenteral therapy with B complex. The recent report of Jolliffe and his collaborators (41) on the response of acne to therapy with pyridoxine is of significance in this connection.

A study by Heilig and Kantiengar (42) on variations in liver function during the menstrual cycle may have a bearing on the reciprocal relation of estrogen and the vitamin B complex. These investigators found that in women in whom there is a relatively low liver function as measured by the Quick test on the 13th or 14th day of the menstrual cycle, there is a significant and further diminution in liver function on the first day of menstruation, *i.e.*, after the two-week period during which the peak or peaks of estrogen occur. In women who had excellent liver function in the mid-interval there was little change on the first day of bleeding. The percentage of decrease in liver function was greatest in those cases in which liver function at the mid-interval was lowest. In view of the known relation of the B complex to liver function, it seems not improbable that the first group mentioned had nutritional deficiency.

Four of the cases reported in this paper had had chronic vaginitis of long-standing; in 3 of these trichomoniasis was present. Vaginitis is a not infrequent occurrence in pellagra (31) and in less severe deficiency states (43). Shute (44, 45) has pointed out that in some patients with vaginitis and vulvitis, there is an excess of estrogen in the blood; at least one of the patients among those he has reported had a definite vitamin B deficiency with severe peripheral neuritis and mental depression. The exacerbations which occur in vaginitis, especially in trichomonas vaginitis, during the mentrual periods, may be related to depression of an already depleted vitamin B reserve by the high body estrogen preceding the onset of menstruation.

Two important features appear in this study. One was that several of the patients first developed syndromes related to an excess of estrogen immediately following a pregnancy, during which time nutritional demands are increased but rarely met (46). Three of these women, significantly, had a subsequent subinvolution of the uterus. The other feature was the relation of these syndromes to obesity; most of the patients were overweight. One patient in particular, age 28, 61 in. tall, weighed 206 lb. Owing to psychogenic factors, during times of family stress she would eat voraciously and gain as much as 50 lb. in a few months. Each time she gained weight, and there were three such episodes, severe menorrhagia and metrorrhagia developed. When she dieted and lost weight the excessive bleeding stopped. During the last bout of over-eating, the bleeding was so severe that she was treated with radium.

It is now well known that the modern 'normal' American diet is generally a deficient diet (47, 48), especially with regard to the B vitamins. Since the need for thiamin, for instance, is related directly to the caloric intake, it is easy to see that, in a diet in which there is less thiamin per 1000 calories than the minimum need, the greater the caloric intake the greater the thiamin deficit. In the cases reported in detail in this paper, the vitamin B complex was administered without any measures directed to reduction in weight. In others now being studied, both therapeutic measures are being employed concurrently.

The need for the B vitamins is also related to the metabolic rate (49). It is significant that in *case 2*, in which a severe nutritional deficiency was present, repeated attempts to administer thyroid because of a low basal metabolic rate led only to exacerbation of the symptoms of nervousness, irritability, insomnia and headaches. The increased metabolic rate thus induced enhanced an already severe nutritional deficiency. In this case also, a further point is of interest. Oral administration

of riboflavin (with the rest of the B complex) over a period of several months failed to affect the cheilosis in any degree although an accompanying glossitis and stomatitis healed rapidly. When thiamin alone was given parenterally, while the remainder of the complex was given only by mouth, there was prompt and definite improvement in the cheilosis. This may be related to the observation by Sure and Ford (50) that in thiamin deficiency there is a disturbance in riboflavin metabolism owing to failure of absorption.

It may appear paradoxical that the mastitis was successfully treated in *case 7* (and with somewhat less success in *case 6*) at one time by giving estrogen and at another time by therapy that might be expected to diminish the body estrogen. However, Gardner (51) has shown that while moderate doses of estrogen in animals stimulate mammary growth, larger doses depress it.

The B complex preparations used orally in this study were usually given in doses providing daily from 3 to 9 mg. of thiamin, from 4.5 to 9 mg. of riboflavin, up to 60 mg. of niacin and niacin amide. The necessity for adequate dosage of vitamin B complex preparations in the treatment of deficiency states cannot be too strongly emphasized. And, as failure of absorption occurs frequently in B complex deprivation, adequate parenteral therapy is often necessary.

It is of interest in connection with the observations reported in this paper that empirically, liver extracts have been used in the treatment of menorrhagia and metrorrhagia (52, 53). It seems likely, from unpublished observations on animals, that the liver fractions owe their effectiveness in pathologic uterine bleeding to their activity in restoring the estrogen-inactivating function of the liver, impaired by nutritional deficiency.

SUMMARY

Evidence is presented that menorrhagia, metrorrhagia, cystic mastitis, pemenstrual tension and possibly other syndromes related to an excess of estrogen, are caused by failure of the liver to inactivate estrogen because of deficiency of the vitamin B complex. Administration of the B complex in adequate dosage orally, parenterally, or by both routes, led to prompt improvement in these conditions.

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of

Effect of Androgens upon Libido in Women

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DURING the course of the therapeutic administration of androgens for various endocrinopathic gynecologic disorders during the past five years, the observation was made that libido was apparently increased in a number of the women being treated (1). Our attention was first drawn to this phenomenon in 1937 by an ovariectomized woman, suffering from the menopause syndrome, who was being treated experimentally with testosterone propionate. During the course of treatment the patient volunteered the information that she had experienced resurgence of sexual desire after a period of quiescence of some ten years. Since then we have been conducting a series of studies to determine the effect of various sterol sex hormones upon the psychosexual reactions of women.

METHODS AND MATERIALS

The present report is based on our observations of a series of 101 women comprising the following groups. *Group A*. Twenty-nine cases of primary frigidity which were being treated for some apparently unrelated gynecologic disorder: dysmenorrhea, 6 cases; menometrorrhagia, 16 cases; polymenorrhea, 3 cases; premenstrual tension, 4 cases. *Group B*. Thirty cases of secondary frigidity occurring *a*) at the menopause, 13 cases; *b*) after bilateral ovariectomy, 7 cases; *c*) after roentgen-ray castration, 7 cases; and *d*) in association with amenorrhea, 3 cases. *Group C*. Forty-two were patients with apparently normal libido who were being treated with androgens for one of the following complaints: dysmenorrhea, 12 cases; menorrhagia, 27 cases; and premenstrual tension, 3 cases.

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Types of androgen therapy and dosage used. Fifty-six of the patients were treated with testosterone propionate in sesame oil intramuscularly in doses of 10 or 25 mg. two or three times weekly, the total weekly doses varying from 20 to 75 mg., for periods varying from 6 to 8 weeks; 22 with methyl testosterone in daily doses of 10 to 30 mg. orally for periods varying from 3 to 6 months; 23 received subcutaneous implants of 2 to 8 pellets of testosterone, each weighing 75 mg. Periods of observation following therapy varied from 8 to 38 months.

RESULTS

Group A. Primary Frigidity with Somatic Gynecologic Disorders

Of the 29 cases in this group 21 were treated with testosterone propionate intramuscularly and 8 had pellet implants of testosterone. Of the 21 cases treated with testosterone propionate, 4 reported excessive sexual stimulation. These patients were conscious of a sensation of formication in the vulva which was most marked in the region of the clitoris. The clitoris and prepuce were hypersensitive and in two cases, slightly hypertrophied. Ten of the cases reported complete sexual gratification, 4 reported considerable improvement but were unable to achieve an orgasm and 3 reported no improvement.

Of the 8 cases with testosterone pellet implants 2 reported excessive stimulation (600 and 525 mg., respectively); 4, normal sexual reactions (225 to 450 mg.); and 2, no response (150 and 300 mg.).

Of the 5 failures in the group of 29 cases, 2 could be attributed to marital incompatibilities. In the remaining 3 there was a heightened clitoridal sensitivity without, however, consonant emotional response. These 3 individuals appeared to be normal, well-in-

formed, well-adjusted women who professed to be, otherwise, happily married.

Group B. Secondary (Endocrinopathic) Frigidity

In order to evaluate the effect of androgens in the presence of an estrogen deficiency the 30 patients with secondary or endocrinopathic frigidity were divided into three groups and treated as follows: *a*) one group of 10 received androgens; *b*) another group of 11, estrogens, and *c*) the remaining 9 cases, androgens and estrogens simultaneously.

Effect of androgens in estrogen-deficient cases. After periods of treatment varying from 4 to 8 weeks, the total doses varying from 240 to 600 mg. of testosterone propionate, 9 of the 11 patients in this group reported increased libido and increased clitoridal sensitivity, but did not experience coital gratification. One patient reported excessive stimulation and one none at all. The 9 stimulated patients stated that coitus could not be satisfactorily consummated because of dryness and tenderness of the vagina. Following the course of androgens, estrogens were substituted for the testosterone without the patient's knowledge (2.5 mg. of estradiol dipropionate three times weekly). After treatment with estrogens for 10 to 14 days, the patients reported disappearance of the vaginal dryness and tenderness and they were able to perform coitus satisfactorily. This improvement in the subjective vaginal reaction paralleled the appearance of a characteristic estrogenic effect in the vaginal smears.

Effect of estrogens in estrogen-deficient cases. Twelve patients were treated with estrogens (2.5 mg. of estradiol dipropionate three times weekly) until the vaginal smears showed a full estrogenic effect, and the vasomotor symptoms were relieved. Libido was not, however, appreciably affected. Eight patients of this group who, prior to the estrogen therapy, had experienced dyspareunia caused by 'senile' vaginitis and who had in consequence developed a distaste for coitus, were relieved of the coital discomfort after treatment with estrogens, but none of these reported a resurgence of libido. At this point, without their knowledge, androgens were substituted for the estrogens (25 mg. of testosterone propionate 2 or 3 times weekly). After approximately 3 weeks, 4 patients reported restoration of normal sexual reaction; 2, excessive stimulation; 1, slight, and 1, no improvement.

Effect of combined estrogen and androgen therapy in estrogen-deficient cases. The 9 patients in this group were given estrogens and androgens simultaneously, 1 to 5 mg. of estradiol dipropionate in combination with 10 or 25 mg. of testosterone propionate two or three times weekly. In 7 instances, the patients reported a marked improvement after 3 or 4 weeks of treatment. The optimal therapeutic effect was obtained in these cases when the vaginal smear revealed a full estrogenic effect even though the androgens were being given. If sufficient androgen, but insufficient estrogen was given, the patient experienced the stimulating effect of the former but coitus was unsatisfactory and a vaginal orgasm was not achieved, because of the dryness and irritability of the vagina; when, however, an adequate amount of estrogen but an insufficient amount of androgen was given, the vaginal dryness and irritability were relieved but libido was not stimulated.

Group C. Cases with Somatic Gynecological Disorders and Normal Libido

Of the 42 patients in this group 10 were treated with testosterone propionate, 25 mg. twice weekly or 10 mg. three times weekly intramuscularly; 12 with methyl testosterone orally, 20 to 50 mg. daily. Fifteen received implants of testosterone pellets, 75 mg. each, total doses varying from 300 to 600 mg.

Results with testosterone propionate. Of the group treated with testosterone propionate, all but one experienced increase of libido after receiving approximately 200 to 300 mg. With this dose a mild stimulation occurred which persisted for several weeks. Continued administration of the testosterone propionate resulted in a progressive increase of libido, in 4 instances to a distressing degree.

Results with methyl testosterone. Methyl testosterone produced moderate stimulation in doses of 20 and 30 mg. a day after treatment for 2 to 3 weeks. In 6 instances doses of 40 and 50 mg. a day caused excessive stimulation

after two weeks of treatment and it persisted for approximately two weeks after discontinuation of the hormone.

Results with pellet implantation. Of the 15 cases in this group receiving pellet implants of testosterone, 3 reported no change (150 mg., 2 cases; 300 mg., 1 case); 5 were moderately stimulated for 4 to 5 weeks (150 mg., 1 case; 225 mg., 1 case; 300 mg., 3 cases). Seven were excessively stimulated for 6 to 8 weeks (450 mg., 3 cases; 600 mg., 4 cases). Subjective evidence of stimulation was reported in 10 of the 12 responsive cases approximately 12 days after the implantation; in 2 cases implanted with 150 mg. increased libido was not noted until approximately 18 days after the implantation.

DISCUSSION

It appears from this study that androgens have a triple action upon the psychosomatic sexual mechanism, causing, *a*) a heightened susceptibility to psychic stimulation; *b*) increased sensitivity of the external genitalia, particularly of the clitoris and *c*) a greater intensity of sexual gratification.

It appears, furthermore, that in the presence of an estrogen deficiency, androgens in the doses used may stimulate the psychic and the sensory components but that culmination in a vaginal orgasm may not be accomplished without the complementary stimulative effects of estrogens upon the vagina. The estrogens cause an increase in the vascularity of the vagina and furnish the physiologic lubricant resulting from the desquamation of the cornified epithelial cells and the cervical secretion which is essential for normal coital sensibility.

It is noteworthy that in 6 of the 13 cases which failed to respond to the androgens, there appeared to be some psychic disturbance to which the frigidity could be attributed. It would seem from these studies that types of frigidity that are predominantly psychogenic in origin are not responsive to androgen therapy. However, the stimulating effects of the androgens are so striking, that even psychogenic types might be benefited, providing one can be reasonably certain that the stimulation would not *per se* cause undesirable psychic repercussions.

The question arises as to whether this stimulating action of the androgens upon the psychic and somatic components of the sexual mechanism should be considered as a pharmacologic phenomenon or whether one may infer from these observations that in the normal female some endogenous androgens may have a similar effect, acting as a physiologic stimulant or activator of the psychic and somatic components of the sexual mechanism. For several years we have fostered the theory that androgens play an important rôle in the steroid sex hormone physiology of the human female, modifying and balancing the action of the gynecogenic hormones (1, 2, 3). We have based the rationale for the use of androgens in the treatment of certain types of menometrorrhagia (4, 5), dysmenorrhea (6), and premenstrual tension (1, 7) upon this theory. It appears from the studies herein reported that the endogenous androgens in the mature human female may have still another function, namely, to sensitize both the psychic and somatic components of the sexual mechanism so that the individual is emotionally receptive to excitation and somatically sensitive to sexual stimulation.

SUMMARY AND CONCLUSIONS

The effect of androgens upon libido was studied in a group of 101 women who were being treated for some endocrine disorder. The androgens were administered in the form of *a*) testosterone propionate in solution in sesame oil, intramuscularly; *b*) pellets of testosterone, implanted subcutaneously; and *c*) methyl testosterone, orally. All but 13 of the 101 treated women reported some increase in libido; 20 noted excessive stimulation which subsided within 2 to 4 weeks after discontinuation of the androgen therapy. From these studies it appears that androgens have a threefold action, causing *a*) an increased susceptibility to psycho-sexual stimulation; *b*) an increased sensitivity of the external genitalia and *c*) a greater intensity of sexual gratification.

It is suggested on the basis of these observations that endogenous androgens in the normal

mature woman may act as the physiologic sensitizer of both the psychic and somatic components of the sexual mechanism.

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Personality Changes in Endocrine Disorders

With a Note on 'Symptomatic Hypoglycemia'

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PERSONALITY may be defined as a resultant of internal forces within an individual which affect his relationship to his environment. It may be regarded as a subjective concept and be subdivided into such components as impulse, temperament, character and intelligence[1] (1). It is a form of 'potential energy,' a summation of attributes which can be brought to bear in the individual's dealings with the world. When it is expressed and becomes kinetic or active, one may designate it as behavior, an objective concept. To evaluate properly the endocrine glands as active causes or even as precursors of personality change, it is necessary to understand the mechanisms involved in any disease-personality relationship. These are the structural, the functional and the psychogenic.

Structural Mechanism

The most fundamental aspect of structure is the constitutional make-up of the individual. His hereditary factors express themselves in certain body types (pyknic or leptosomic) with a resultant predisposition toward one or another form of personality or mental disease (manic-depressive or schizophrenic). Various other classifications of constitutional types have been evolved, but it is just as wrong to explain all disease entities from the constitutional viewpoint as to ignore this fundamental subject. An intercurrent disease, of whatever nature, with its personality alteration must consequently be considered in the light of the inherent constitution which is the oldest and most primitive determinant of the personality pattern throughout life. Disease will not affect the underlying constitution but there is evidence that certain types of constitution may predispose to both physical and psychic disorders. Another type of organic or structural factor is found in the congenital anomalies. These somatic defects may originate in the germ plasm or arise during intrauterine existence. Anomalies in brain development fit into this category. They may cause profound personality abnormalities which are practically irremediable or may be so slight as almost to escape notice. Diseases such as mongolism, Laurence-Moon-Biedl syndrome and Turner's syndrome are examples of this type. A third type of organic mechanism which alters the personality pattern is represented by the pathologic changes incident to cerebral hemorrhage, infections and tumors. Endocrine manifestations are often observed concomitantly with these organic factors, since the pathologic process in the brain may eventually involve the pituitary and/or the hypothalamic centers. A whole chain of abnormal endocrine phenomena may thus be set in motion. Personality changes frequently observed early in patients with gigantism or acromegaly are examples of this type.

Functional Mechanism

The second type of mechanism producing personality alterations is purely functional. It is also called symptomatic. Being due to altered biochemical or autonomic-nervous activity, it is most often reversible. For this reason, its

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[1] The concepts of personality advanced by Kahn, based as they are on clinical descriptive methods, lend themselves ideally to clinical medicine. Adaptable to simplification as undertaken here, it offers to the internist an inclusive and common-sense approach to the study of personality.

early recognition is important for the welfare of the patient. The toxic psychoses from acute infections, drugs and alcoholism are here represented. Alterations in the female psyche with the menarche, menstruation, pregnancy and the menopause are similar examples. Endocrine disorders in general affect personality most frequently through this functional mechanism. They do so primarily through an increase or decrease of the impulse and emotional tension. The effect is non-specific in the sense that it swings mood or tempo in either direction. The hyperkineses and increased drive with or without irritability are seen in hyperthyroid patients and their converse in hypothyroidism. Similarly, the gonads appear primarily to influence impulse and emotional tension. Superimposed psychogenic reactions due to difficulties in coping with the environment and even major psychoses (manic depressive or schizophrenic) may be precipitated as secondary effects.

A frequent functional mechanism affecting personality reactions and one found with or without endocrine disease, is hypoglycemia. In this condition there occurs a depression of the blood-sugar level sufficient to interfere with the normal functions of the nervous system and to lead to a variety of distressing symptoms such as apprehension, a feeling of insecurity, clouding of consciousness, loss of attention, asphasia, irritability and fatigue. In other words, the higher cortical functions may at times be disturbed leading to temporary abnormalities in impulse, temperament, character and intelligence. In addition, unpleasant neurologic signs may appear, ranging in their mild forms from diplopia, sweating and tremor to the more rare and severe types with convulsions and coma. The mild forms are extremely common and occur in many metabolic and endocrine disturbances. In fact, the symptoms often occur in the absence of any known disease process. Because such phenomena assume a leading place in the relationship of personality complaints to clinical practice, several examples are cited.

CASE REPORTS

A nurse, age 23, became apprehensive, tremulous and confused during her ward work at 11 A.M. Treatment with sedatives gave no relief. The condition persisted for 6 months, leading to distress and worry and a consideration of changing her occupation. She was in the habit of eating a high carbohydrate-low protein breakfast at 7 A.M. Regulation of the diet to augment the protein intake at breakfast with a small carbohydrate supplementation at 10 A.M. controlled the symptoms and prevented the attacks.

A girl, age 12, experienced confusional states followed by fainting which usually occurred before noon on Sundays when she was in church. She was accused of malingering and dealt with severely by her parents. Breakfast on these days was unusually light and very early. The attacks promptly subsided when proper dietary steps were taken.

A physician's daughter, age 16, was brought in with a possible diagnosis of pituitary tumor because of visual complaints during school at about 11 A.M. Several times a month she would be brought home, aphasic and put to bed without food because of nausea and headache (a frequent hypoglycemic symptom) until suppertime when, following eating, she would improve. As occurs so frequently, the episodes were aggravated during the week preceding menstruation. All symptoms were promptly and completely controlled with dietary regulation.

A dentist, age 36, developed a habit of avoiding difficult work between the hours of 10 A.M. and noon because he found the results to be of an inferior quality, and work done at such time frequently had to be repeated. He consulted several doctors and a psychiatrist. He abandoned smoking and relinquished some evening social activities, but to no avail. Here again dietary regulation solved the problem completely.

Relatively simple cases uncomplicated by concomitant clinical disease have been presented. They are characterized by: *a*) a predisposing type of dietary history, namely, high carbohydrate and low protein (or non-protein) meals, or long intervals between meals; *b*) evidence of autonomic vascular instability; *c*) failure to respond to sedation or other medication; *d*) cure by dietary means. All responded to an increased protein intake at meal time (at the expense of carbohydrate) plus the addition of a single small carbohydrate supplementation between meals. From the laboratory standpoint these cases are frequently obscured because the blood sugar at the time of complaint (as well as on the 4th and 5th hour of the glucose tolerance test) may be normal or at best only moderately depressed. The blood sugar does not fall to the low levels characteristic of hyperinsulinism in pancreatic adenoma,

in the excessive insulin sensitivity of some endocrine disorders (Simmonds' disease or Addison's disease) or as in liver disease. In contrast to this true organic hypoglycemic group, the cases here cited are described under the term of 'symptomatic hypoglycemia,' because the blood sugar values, at the time of complaint, cannot be demonstrated to be below the accepted normal. The phenomenon depends in final analysis less upon the level of the blood sugar than upon deficient carbohydrate oxidation in the central nervous system. This is in turn influenced by the adequacy or inadequacy of cerebral circulation, the oxygen supply, and the sensitivity of the various parts of the central nervous system itself both to diminished oxidation and to any compensatory adrenalin response. These factors account for the frequency of 'symptomatic hypoglycemia' in all clinical cases of neurovascular autonomic instability. The cases cited are but a few of many that we have seen. When uncorrected, the conditions may lead to distressing emotional and mental reactions which interfere with efficiency and produce anxiety states. The matter deserves far greater attention than it commonly receives in schools, colleges and factories where food consumption is hurried and inadequate, where hours are long, and emotional and mental tension runs high.

Psychogenic Mechanism

The third fundamental mechanism of personality aberration is the psychogenic. In all such cases the mechanism is set in motion by an awareness of the problem, be it external (environmental) or internal, such as the knowledge of disease or bodily abnormality (somatopsychic). Every disease process is accompanied or followed by some psychogenic reaction. The personality disturbance may be superficial and easily overcome by the correction of the disorder. It may become deep-seated into a pattern reaction or a personality habit that persists long after the exciting cause is withdrawn. It may become intense enough to obscure completely and over-shadow the precipitating etiology. It may even precipitate or act as the trigger mechanism in the production of a major psychosis.

Since bodily aberrations of which the patient is aware are common in endocrine practice, one might expect pronounced somatopsychic inferiorities. Such is indeed the case. The problems raised by obesity, hirsutism, hypogenitalism and dwarfism from the purely somatic angle demonstrate this well (2). One child may become extremely retiring and anti-social and the next one may compensate in the opposite direction. A somatopsychic mechanism of this type operating over a period of years, produces definite personality habits which may persist long after the original cause has been removed. One must not overlook that portion of the psychogenic mechanism caused by the environment. It usually consists of a long-standing psychologic problem of which the patient may or may not have been fully aware. The victim of his unsolved problems may not present any evidence of mental stress until a critical period in life when physical ill health precipitates some acute manifestation. For example, a woman who has been unhappy most of her life with an incompatible husband (environmental stress) turns to alcoholic excess at the time of menopause (added psychologic stress). It would be an easy matter to explain the situation in terms of menopause alone. Such an explanation, however, is not adequate. It was psychologic trauma of long-standing precipitated into an intolerable situation by a functional mechanism (menopause) which led her to attempt escape by the use of alcohol. In such cases proper estrogenic substitution may relieve the menopausal symptoms but will not get rid of the husband or control the alcoholism. However, a common-sense psychotherapeutic explanation of the entire mechanism may contribute further to a solution of the problem.

SUMMARY

The triad of mechanisms, structural, functional and psychogenic, operate to produce personality changes. They may operate singly or more often in combination. Every medical case and particularly every endocrine case, presents problems in personality mixed with the medical story. They can almost invariably

be brought into the clear by skillful questioning. The personality factors may be represented only as a side issue or they may become the major complaints obscuring the entire clinical picture. Concomitantly with clarification of the medical aspects of any clinical, and particularly, any endocrine problem, it is essential to visualize the personality or behavior pattern in the light of the medical information obtained. Medication alone does not solve these problems, nor does the application of any popular psychologic formulation.

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Furunculosis—Etiology and Treatment

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EVIDENCE indicates that there are many factors in the etiology of furunculosis. There seems to be a widespread belief among laymen that boils are the result of 'bad blood,' but no scientific support has been presented to substantiate such a view. Cleanliness of the skin was considered a major factor by Gant, Owens and Schwartz (1) in a severe outbreak of boils among a group of tunnel workers. The resistance of the host is an important factor in any infection and it seems logical to assume that low tissue resistance would play a rôle in the development of furunculosis. The present report reviews 16 cases in which tissue resistance seemed to be an important factor.

METHODS

The 16 patients were college students between the ages of 17 and 25 years. They represented the only cases of furunculosis seen among approximately 2000 students over a period of one year. A careful history was taken and a physical examination made; particular attention was given to evidence of dietary deficiency. Hemoglobin was estimated by the Newcomber method. The basal metabolism was determined using the usual precautions and the new Sanborn waterless apparatus. Basal temperature was obtained by the method previously described in which the thermometer is left under the tongue for 10 minutes before the patient gets out of bed in the morning. Oral and rectal temperatures were taken at the time of examination.

RESULTS

No correlation was apparent between the hemoglobin values and the development of boils. The average was 14.0 gm. of hemoglobin per 100 cc., the range 11.5 to 17.0 gm. Many students free from boils have been seen in whom the hemoglobin values were in the lower part of this range. The largest boil observed in this group of patients was in the axilla of a boy whose hemoglobin was 15.3 gm.; the basal metabolic rate in this case was minus 24.

TABLE 1. BASAL TEMPERATURE AND B.M.R. IN 16 CASES OF FURUNCULOSIS

Patient	Temperature °F.	B.M.R.	Sex
MH	97.0	−12	M
JH	97.0	−24	M
DC	97.2	−19	M
BS	97.0	−20	M
CT	97.0	−19	M
MJ	97.4	−13	M
AG	97.5	−10	M
BS	97.6	− 5	M
DC	97.5	− 9	M
WS	97.5	+ 7	M
GJ	97.0	−20	M
JO	97.0	−15	M
DD	97.4	− 9	F
GM	97.6	−15	F
AG	97.4		F
GM	97.2		F

The history and physical examination disclosed no evidence of malnutrition in this group of patients. There were no obvious local skin conditions contributing to the development of furunculosis. One factor was common in the physical examinations, namely, subnormal temperatures (table 1). Temperatures taken at the time of physical examination were unreliable since excitement or exercise will elevate the temperature. However, in most people with a subnormal temperature the rectal temperature is below 99.0° F. at the time of examination. Basal temperatures were uniformly low on this group of cases. The normal range for basal temperature is thought to be about 97.8 to 98.2° F.

The determinations of the basal metabolic rate were low, with one exception in which the reading was plus 7. However, in this case the body temperature was low and the high basal

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metabolic rate was thought to result from nervousness and inability to relax. Most of the basal values were between minus 24 and minus 10 on the first test (table 1). It is thought that all cases would have been in this range if repeated determinations had been obtained. However, basal temperature is now being used as a criterion of lowered tissue resistance more than basal metabolism.

Therapy. The furuncles were given local treatment of heat and incision for drainage after pus was apparent. A few healed by resorption without being opened. Heat therapy consisted of hot wet dressings at home and 30 minutes to one hour under a Zeolite infra-red lamp at some time during the day at the Health Department.

Two cases served as controls without systemic therapy for 2 to 3 months. Boils recurred during this period; heat therapy was given as often as the time of these patients would permit but in each case the furuncle would enlarge and require incision. In one of the control cases thyroid therapy (1 grain a day) was started and heat therapy stopped when there were two tender lumps under one arm and one under the other. These were about half the size usually seen in this patient at the time of incision. Within 10 days the three tender lumps had completely disappeared, and no evidence of a recurrence has been observed. In the other control case thyroid therapy was started during a period of remission and no further furuncles appeared.

In the remaining 14 cases of furunculosis thyroid therapy was started when the patient was first seen. A second boil did not develop in any case during the period of thyroid therapy. One student who had repeated boils before therapy was started had no recurrences during 8 months of treatment. He then stopped medication without medical advice and boils recurred within two months. Details of the results with continued therapy have been published (2).

DISCUSSION

In the study of furunculosis in the present series it would seem that most of the usual etiologic factors, including cleanliness of the skin, can be excluded. The low metabolic rates and subnormal temperatures indicate that tissue resistance might be low. Experimentally it has been shown that skin disorders are numerous in the cretin rabbit (3). In cases of human myxedema the blood flow per minute and the skin temperature are markedly reduced and are promptly restored to normal during thyroid medication (4). Since the healing process is accompanied by an increase in circulation, it is not surprising that thyroid therapy would aid in skin infections in cases with poor peripheral circulation. Furthermore, it seems probable that an increased flow of blood through the skin would aid in the prevention of localized infection. The prompt response to thyroid therapy of the furuncles in this series of cases seems to furnish evidence for such a theory. The resistance of the skin to local infection seems to have improved since no more furuncles developed during the period of thyroid therapy. It is impossible to state whether the resistance was due to an action on the individual cells, to an increased blood flow, or to the combination of the two. Ball (5) reported that 'boils cleared with dramatic suddenness' using thyroid and potassium permanganate. It is impossible to state how much of the success with this therapy was due to both constituents but the same results have been obtained in the cases reported here with the use of thyroid alone.

SUMMARY

Furunculosis was observed in 16 patients. The basal metabolic rate or basal temperature was below normal in each case. Further lesions did not develop after starting thyroid therapy, unless the medication was discontinued. It would appear that skin resistance may be a factor in furunculosis if the peripheral blood supply is below normal.

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EDITORIAL

AN ANTI-THYROID PITUITARY HORMONE

In his dependence upon the regulatory influences of the endocrine glands every human being is subject to two hazards. One is that his various endocrine structures will fail to rise to adequate heights of activity and the other is that they will become too active. When these possibilities are realized we have, on the one hand, such conditions as myxedema or simple amenorrhea and on the other hand, such disorders as exophthalmic goiter or von Recklinghausen's disease.

To the clinical endocrinologist, the second category of disorders is at least as impressive as is the first, but laboratory investigators have in effect largely confined their attention to the first. This comes about perhaps because experimental animals seldom appear in the laboratories in a condition of glandular hyperactivity. It is customary, therefore, to think of problems of glandular control mostly in terms of presence or absence of stimulation. The general problem of why we do not all develop fatal exophthalmic goiter or other glandular hyperactivity early in life has received comparatively little explicit study. We are wont to think of the matter in about the same terms as those in which we regard the problem of control of the furnaces which heat our homes. When more heat is desired the drafts are opened and the fire burns more briskly. When the rooms become too warm the drafts are closed and the fire dies down. It is only when a conflagration breaks out that positive measures for the lessening of combustion are invoked. The possibility that the glands may be under positive inhibitory as well as stimulative control has received relatively little consideration at the hands of investigators.

In the case of nervous control of body functions, on the other hand, the possibilities of positive and of negative control have been well explored, and the principle of reciprocal innervation has been well established. Thus dilatation of the pupils may be brought about either by stimulation of dilator mechanisms or by inhibition of constrictor mechanisms. Similarly, a rapid pulse may be evoked either by augmented activity of the sympathetic innervation or by depression of the vagus mechanism.

In a recent publication from the Institute of Physiology of the University of Buenos Aires, Reforzo-Membrives[1] has approached the problem of thyroid control in terms of inhibition rather than of stimulation. The strategy of his study was based on the fact that the anterior pituitary of the rat exerts a strong thyroid-stimulating action and the thyroid of that animal is highly resistant to such stimulation whereas the thyroid of the guinea pig is very sensitive to the action of thyrotropic hormone. By utilizing both species jointly in the research it was possible to exploit both the high efficiency of a donor gland and the high sensitivity of a recipient organ.

To adult male rats desiccated thyroid substance was given in daily dosage of 50 to 100 mg. for periods of 22 to 32 days. The hypophyses of the treated rats were then administered intraperitoneally in saline-fluid suspension to young guinea pigs. Usually 2 glands were given on each of 2 successive days. The results of the treatment were then determined, using three criteria: the thyroid glands of the recipient animals were studied histologically, the oxidizing power of the same glands was determined, and the basal metabolic rates of the treated guinea pigs were investigated. The studies were controlled by the use of hypophyses from rats kept on standard diets without the thyroid administration.

It was found, in brief, that when normal rat hypophyses were injected, the expected thyrotropic influences appeared. The thyroids of the treated guinea pigs were 35 per cent *heavier* than those of untreated animals and showed the typical picture of hyperfunction. But when the hypophyses of thyroidized rats were used, the opposite sort of effects was noted. The thyroids of the recipients were 26 per cent *lighter* than normal and showed a corresponding reduction in the height of the thyroid epithelium. Similarly in the first case the oxidation index of the recipient thyroids was 28 per cent augmented while in the second case it was 23 per cent decreased. Finally, the basal metabolic rate of the guinea

[1] Reforzo-Membrives, J.: Thyroid-inhibiting action of the hypophyses of rats fed with thyroid. *Endocrinology* 32: 263. 1943.

pigs was augmented from 9 to 25 per cent by the normal rat hypophyses but depressed from 26 to 48 per cent by the hypophyses of thyroidized rats. In control experiments in which tissues other than the hypophyses of such rats were administered, the thyroids of the recipient animals were not significantly affected.

The evidence thus seems clear that when the organism is subjected to hormone over-stimulation not only may there be a depression of autogenous hormone which would exaggerate the functional pathology but also secretion of a positive antidoting hormone. These new findings supplement in a meaningful way the older observations that gland deprivation results in the augmentation of compensatory stimulating hormone as is seen, for example, when the hypophyses of castrated animals secrete excessive amounts of gonadotropic hormone. Thus a principle of reciprocal hormonal control comparable with that of reciprocal innervation is exemplified. A research investigation of such fundamental significance will no doubt stimulate similar studies in other laboratories and with other species.

The findings of Reforzo-Membrives offer a suggestion as to how iodine, which primarily augments thyroid secretion, can be effective in mitigating the symptoms of exophthalmic goiter —a paradox that has long demanded elucidation. Possibly the iodine administration results, directly or indirectly, as does thyroid administration, in the production of hypophyseal antithyroid hormone. It may be that it is this pituitary hormone which was, in whole or in part, the effective agent in the 'anti-thyroid serum' of older investigators and in animals 'immunized' by thyroglobulin as recently studied by Lerman.[2]

The demonstration of a specific depressive hormone in case of thyroid-treated rats demands further search for similar agents in other cases of induced or clinical conditions of hyperhormonization. Should a principle comparable to reciprocal innervation prove to be of general occurrence in our field, endocrine theory would be fundamentally enriched and a way might be opened for an effective extension of the possibilities of hormone therapy.

R.G.H.

[2] LERMAN, J.: Endocrine action of thyroglobulin antibodies. *Endocrinology* 31: 558. 1942.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: DANIEL A. MCGINTY. *Collaborators:* ISRAEL BRAM, JOHN C. BURCH, JOHN C. DONALDSON, J. W. EVERETT, MURRAY B. GORDON, R. B. GREENBLATT, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, J. E. HOWARD, J. T. LEWIS, T. H. MCGAVACK, A. E. MEYER, C. C. PFEIFFER, J. P. PRATT, E. C. REIFENSTEIN, BORIS B. RUBENSTEIN, PATRICIA H. SMITH, EMMERICH VON HAAM, HAROLD WOOSTER.

BOOK REVIEWS

The Biological Action of the Vitamins. A Symposium. Ed., E. A. Evans, The University of Chicago Press, Chicago, Ill. 1942.

The hormones and the vitamins, although separately categorized, are biological regulators of a similar order. Both have important influences on cellular metabolism and deficiencies of both are manifested in subtle changes in nutrition as well as in physiological functions. Both likewise are characterized by remarkable potency, functioning perhaps in all cases as catalyzers or components of catalytic systems. The interrelations of the vitamins and the hormones are increasingly engaging the interests of investigators. Although this book is not primarily concerned with the hormones it will be of interest to all endocrinologists who are concerned with underlying biology.

The first article, by C. A. Elvehjem deals with the biological action of the vitamins. It is in the nature of a historical introduction. It includes a section on the functional relations of the vitamins and the hormones. This is followed by a discussion of vitamins and enzymes and their interrelationships.

Thirteen other chapters by outstanding investigators deal with cocarboxylase, vitamine B_1, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, choline, the economy of phosphorus, and vitamin K. The chemical, biological and clinical aspects of each are considered.

The book is readable, authoritative and relatively comprehensive. It can be highly recommended to non-specialists seeking fairly easy orientation in the field.—*R.G.H.*

ADRENALS

CLINTON, M., AND G. W. THORN.

The effect of 11-desoxy-17 hydroxycorticosterone on renal excretion of electrolytes *Science* 96: 343. 1942.

Results of injection of a normal dog with 11-desoxy-17 hydroxycorticosterone showed that it belonged to the group of compounds possessing "Na and Cl-retaining" property. Addition of a hydroxyl group on C_{17} to desoxycorticosterone resulted in formation of a compound with lower sodium and chloride-retaining properties, but similar addition to corticosterone resulted in formation of a compound which facilitated Na and Cl excretion.—*Courtesy Biol. Abst.*

ENGEL, F. L., W. H. MENCHER AND G. L. ENGEL.

"Epinephrine shock" as a manifestation of a pheochromocytoma of the adrenal medulla. Report of a case with successful removal of the tumor. *Am. J. M. Sc.* 204: 649. 1942.

The successful removal of an adrenal medullary pheochromocytoma in a 23 year old woman is recorded. Symptoms resembling epinephrin shock had been present in increasing severity for 2½ years. Shock attacks were precipitated by any stimulus which elicited sympathetic discharge. Postoperatively the patient developed a transient postural hypotension and a low sugar tolerance curve with hypoglycemia.—*C.P.*

HOFFMAN, W. C., R. A. LEWIS AND G. W. THORN.

The electro-encephalogram in Addison's disease. *Bull. Johns Hopkins Hosp.* 70: 335. 1942.

Abnormalities in the resting pattern of EEG and increased sensitivity of EEG to voluntary hyperventilation observed in high proportion of patients with Addison's disease, are unaffected by desoxycorticosterone acetate. Adrenal cortical extract and intravenous glucose in some instances reduced cortical sensitivity to hyperventilation. —*D.A.M.*

KEELE, D. V., AND K. D. KEELE.

The syndrome of haemorrhagic suprarenal infarction. *Brit. Med. J.* 2: 687. 1942.

A case of haemorrhagic infarction of the right suprarenal gland is described in a young woman who was eight months pregnant. Diagnosis was made after post-mortem examination. The clinical picture in this case was compared with that of cases in the literature and it was considered that this rare condition may be diagnosed in life. The following points present a well defined but heretofore unrecognized syndrome: 1. Pain: Gradual onset of pain with a "cold feeling," which in a few hours becomes very severe and is localized over the adrenal. 2. Vomiting: Copious and repeated vomiting is present. The severity is such that intestinal obstruction is considered. 3. Absence of shock: This is a finding which is completely opposed to expectations in a patient with suprarenal disease and with vomiting of the severity mentioned.—*R.B.G.*

TAYLOR, WM. N., AND B. WISEMAN.

Carcinoma of the adrenal cortex in a child one year of age: Case report. *J. Urol.* 48: 38. 1942.

A child who appeared normal except for greatly enlarged abdomen and somewhat hypertrophied scrotum, testes and penis died during exploratory operation. Autopsy showed 400 gm. adrenal cortex carcinoma with moderate hyperplasia of prostate and lymph glands.—*D.A.M.*

THORN, G. W., S. S. DORRANCE AND E. DAY.

Addison's disease: Evaluation of synthetic desoxycorticosterone acetate therapy in 158 patients. *Ann. Int. Med.* 16: 1053. 1942.

Use of doca over a period of 1.5 years was associated with rehabilitation of about 50% of patients with striking reduction in mortality. Complications resulting from overdosage may be greatly reduced by regulation of treatment. One hundred and forty-eight patients were treated by pellet implantation.—*D.A.M.*

WILSON, A.

Sublingual therapy in Addison's disease. *Lancet* 1: 762. 1942.

Sublingual administration of desoxycorticosterone acetate in propylene glycol failed to maintain 4 patients with Addison's disease in doses of 10 mg. daily. Previously 10–20 mg. per week intramuscularly had proven satisfactory.—*D.A.M.*

ENDOCRINE GENERAL

ABRAHAMSON, R. H., R. CHURCH AND J. W. HINTON.

Hormone effects on the male gastroduodenal mucosa. *Am. J. M. Sc.* 204: 809. 1942.

A correlation between age incidence and sex distribution of gastric carcinoma suggests a physiologic effect of the sex hormones on the gastro-intestinal mucosa. Twenty-two males were accordingly treated with estrogens and the following physiological facts were noted. (1) A 40 to 45 unit rise in free acid response after histamine. (2) A marked increase in free and total acid after a gastric test meal. Treatment of male peptic ulcer patients with a daily parenteral injection of estrogens resulted in a higher incidence of 5–10 month remissions than that which occurred in a corresponding control group.—*C.P.*

DAVIDOFF, E., AND G. L. GOODSTONE.

The treatment of involutional psychoses with diethylstilbestrol and estradiol. *Dis. Nerv. System* 3: 358. 1942.

Estradiol was given in routine dosage of 400,000 I.U. the first week, then 100,000 less each week to a maintenance dosage of 200,000 I.U. per week. The periods of treatment ran from 6 weeks to 2 months followed by a tapering-off period in each case. In some cases of relapse the maintenance regimen was repeated. The diethylstilbestrol was similarly given in initial 4 mg. daily dosage tapering to 2 mg. In 45 cases of involutional psychosis treated with these estrogens, 62% improved as compared with 43% in an untreated control series of 176 cases. The two estrogens were about equally effective. In the milder types of the psychosis the results were better than in the more severe types; in the most severe the results were negligible. Five schizophrenics and 5 manic depressives responded poorly. In 15 cases of involutional syndrome without psychosis the symptoms were generally relieved.—*R.G.H.*

FALCONER, M. A., AND C. L. COPE.

Fibrous dysplasia of bone with endocrine disorders and cutaneous pigmentation (Albright's disease). *Quart. J. Med.* 11: 121. 1942.

The authors present 2 new cases of their own and summarize the literature on the syndrome first described by Albright, Butler, Hampton and Smith. They describe the picture as Albright's disease but for a technical name suggest polyos-

totic fibrous dysplasia. The outstanding features of the syndrome are multiple osseous lesions associated with endocrinological disturbances of various types but usually with skeletal precocity; and some have sexual precocity, features suggesting thyroid disorders and acromegalic changes. Patches of cutaneous pigmentation are also characteristic of the disease. In the 2 patients the authors report there were also present visual changes, apparently due to the great bony overgrowth of the base of the skull pressing on the optic nerves and, in one instance, encroaching on the orbit. In 1 patient also the serum calcium was reported elevated to 12.6 mgm.%, the phosphate lowered to 2.6 mgm.% and serum phosphatase high at 30 Kay units. They stressed the absence of osteoclastic foci, which was also stressed by Albright et al., and believed the pathologic picture to be due to collagenous osteogenesis of the marrow with attritive osteolysis of the lamellar trabeculae.—*J.E.H.*

HARRISON, F. G.

Urinary obstruction in children inducing renal hyperparathyroidism. *J. Urol.* 48: 44. 1942.

Urinary obstruction with imposed infection produces renal insufficiency and induced hyperparathyroidism with or without rickets, dwarfism and infantalism being present. 5 cases are presented with mortality in 2. Of 3 postoperative cases, 2 are free of infection and have resumed growth; one has a guarded prognosis with a single kidney and infection. Prognosis in such cases is poor unless treatment is established before renal insufficiency has progressed.—*D.A.M.*

JULIAN, O. C., D. E. CLARK, J. VAN PROHASKA, C. VERMEULEN AND L. R. DRAGSTEDT.

The antagonistic effect of lipocaic and the anterior pituitary on fat metabolism. *Am. J. Physiol.* 123: 264. 1943.

Lipocaic, the factor of the pancreas that prevents deposition of fat in the liver of the depancreatized dog, inhibits the accumulation of fat in the livers of guinea pigs injected with the ketogenic factor of the pituitary gland. Fatty livers observed in the "Houssay animal" are also prevented by lipocaic. The results imply that lipocaic is antagonistic to the fat metabolism factor of the pituitary gland and causes migration of fat to the body depots. However, factors other than the ketogenic hormone of the pituitary gland are likewise involved in fatty infiltration of the liver and are also controlled by the lipocaic activity of the pancreas.—*R.B.G.*

LAWRENCE, R. D., A. MEYER AND S. NEVIN.

The pathological changes in the brain in fatal hypoglycaemia. *Quart. J. Med.* 11: 181. 1942.

The authors briefly review the literature on fatal hypoglycemia and the various conceptions which have been proposed as to the cause of death in this condition. They report 6 cases of their own with fatality resulting from hypoglycemia. Two of these were diabetic patients whose hypoglycemia resulted from inadvertent administration of too great an amount of insulin; 1 patient suffered his hypoglycemia from an unrecognized islet cell adenoma; the other 3 were given insulin shock therapy for mental disorders. The most characteristic pathologic feature of the condition is the widespread degeneration and disappearance of the nerve cells in the cerebral cortex, basal ganglia and cerebellum, especially noteworthy and constant being the changes in the caudate nucleus and putamen. They point out the lack of evidence that insulin has any direct effect on the vasomotor regulation of the brain. They feel that the most likely explanation for the mechanism of the hypoglycemic changes is due to the inability of brain tissue to use any other substrate than glucose for its oxidative processes. Absence of glucose thus means "suspension of vital metabolism which, if prolonged, must lead to death and irreversible lesions in the nervous tissue." The more prolonged the coma from hypoglycemia, the more likely the fatal termination. They also point out that it is incorrect to describe this reaction as anoxia and suggest a new term—oxyachrestia (cf. achrestic anemia, from $\chi\rho\eta\sigma\iota\varsigma$ = use).—*J.E.H.*

MOORE, T. V.

Physiological factors in the treatment of mental disorders. *Psychiat. Quart.* 16: 765. 1942.

The author treated a series of patients presenting in general manic depressive symptoms with an extract of adrenal cortex and a number of schizophrenic patients with anterior pituitary-like substance. The manic depressive patients received one cc. of eschatin 3 times a week, the schizophrenic patients 250 units of follutein 3 times a week. The initial dose of follutein was usually 125 units. After a course of 15 to 20 injections, a new series was started after an interval of a week to 10 days. Five or more series were

given according to the circumstances. Eschatin was supplemented by ascorbic acid, 100 mg. tablet, t.i.d., a.c. Follutein was supplemented by thiamin hydrochloride in the same dosage. There is some weak evidence to show that the treatment of the dementia praecox patients was beneficial, but rather strong evidence that the conditions of the manic-depressives were distinctly ameliorated. The efficacy of the treatment was tested by the comparison of a treated series of hospital patients (24 cases) with an older series (48 cases) of untreated hospital patients. The critical ratio of the difference in the percentage of recoveries in the 2 series was 4.62, which suggests that for some reason other than chance the treated series had a much more definite trend to recovery. Of the untreated patients, 8.3% and of the treated, 58.3% were discharged from the hospital in less than 6 months. Besides the hospital cases 23 ambulant patients were treated, with 69.6% of apparent recoveries. The author feels that this type of therapy merits further trial.—From "*Hartford Abstracts.*"—*R.G.H.*

SELYE, H.

Production of nephrosclerosis by overdosage with desoxycorticosterone acetate. *Canad. M. A. J.* 47: 515. 1942.

In 12 3-day-old White Leghorn chicks, the author gave daily 0.5 mgm. desoxycorticosterone acetate in oil subcutaneously. Controls were treated with similar doses of cholesterol. After 10 days it was obvious that the D.C.A.-treated birds were consuming more water and developing edema. All the birds were killed at the 20th day. Organs of the control group were normal. The treated birds showed signs of fluid retention with ascites, pericardial effusion and the subcutaneous tissues were edematous. Gonads in both sexes showed marked distension with edema. The hearts were enlarged, partially due to hypertrophy and partially to dilatation. Blood vessels were thickened. The kidneys were considerably enlarged and were grayish and swollen. The tubules had distended lumens. There was cloudy swelling in all segments of the nephron down to the collecting tubules. The entire parenchyma showed hyperaemia and the glomeruli were enormously enlarged. The NPN did not seem to be raised despite marked renal lesions. The blood sugar, however, was increased in the treated ones. The possible significance of these observations relative to the pathogenesis of hypertension and nephrosclerosis in man is discussed.—*J.E.H.*

SELYE, HANS, AND S. ALBERT.

Morphogenetic actions of various steroids in the castrate male rat. *J. Pharmacol. and Exper. Therap.* 76: 137. 1942.

The various steroids stimulate the male accessory sex organs in a selective manner. Testosterone stimulates predominately the seminal vesicles, androsterone the prostate, and androstenediol the preputial glands. Kendall's Compound "E" has a definite prostate stimulating activity. Progesterone and pregnenolone have no seminal vesicle stimulating action in castrates. Desoxycorticosterone is without stimulant activity and appears to have inhibiting action due to the atrophy of the adrenal cortex.—*C.P.*

WILLIAMS, E. H., AND C. A. WRIGHT.

Clinical studies of endocrine dosage in the treatment of involutional psychoses. *M. Rec.* 155: 11. 1942.

Eleven cases were treated successfully and without recurrence for at least one year. Treatment consisted of 10,000 rat units α-estradiol benzoate one to three times a week for several weeks or until mental symptoms and general physical condition approached normal. Concurrent use of sedatives was limited to small doses. Authors call attention to importance of adequate dosage in treatment of this condition. —*D.A.M.*

ZEIFERT, MARK.

Massive dose testosterone therapy in male involutional psychosis. *Psychiat. Quart.* 16: 319. 1942.

Five patients were treated with high doses of testosterone propionate, 25 mg. twice daily, one course amounting to at least 1300 mg. over 40 days. Elimination of suggestion was attempted. Only in one case control with sterile water is reported. No urine assays were done. Erections and rare ejaculations occurred, but no priapism was noted. Psychologically: 2 cases were scarcely influenced, 1 showed improvement lasting 2 months, 2 patients improved and have been adjusting outside since 1940. The author attributes the improvement to the hormone therapy.—*Courtesy Biol. Abst.*

ZONDEK, B., AND Y. M. BROMBERG.

Autodetoxication of stilbestrol during pregnancy. *Lancet* 1: 381. 1942.

Clinical observations on 4 women showed that during pregnancy and the puerperium, women

can tolerate very large doses (270–445 mg.) of diethylstilbestrol dipropionate without developing side-effects. The same patients some months after childbirth showed severe toxic manifestations with only 2–5 mg. doses indicating that detoxification of diethylstilbestrol takes place during pregnancy.—*D.A.M.*

GONADS

ALBRIGHT, F., P. H. SMITH AND R. FRASER.

A syndrome characterized by primary ovarian insufficiency and decreased stature. Report of 11 cases with a digression on hormonal control of axillary and pubic hair. *Am. J. M. Sc.* 204: 625. 1942.

Eleven cases are presented as a new clinical syndrome resulting from life-long ovarian insufficiency. This syndrome is characterized by infantile sexual organs, lack of breast development, sparse axillary and pubic hair, short stature, congenital anomalies, late closure of the epiphyses, precocious senility, excess urinary excretion of follicle-stimulating hormone and a decrease but not an absence of urinary excretion of the 17-keto steroids. The syndrome must be differentiated from other conditions causing primary amenorrhea, namely panhypopituitarism, "premenarchal menopause praecox" and a selective deficiency of the gonadotropic hormones of the anterior pituitary. The criteria for this differentiation are outlived. The author suggests that the lack of hair is due to deficiency of the adrenal cortical hormone. Replacement therapy with estrin leads to marked amelioration of the syndrome.—*C.P.*

BISCHOFF, F., AND G. J. CLARKE.

Influence of nephrectomy on ovarian response to gonadotropins. *Am. J. Physiol.* 138: 241. 1943.

Three gonadotropic preparations, sheep pituitary gonadotropin, pregnant mare serum and pregnancy urine extract, were tested in the partially nephrectomized 22 day old female rat. The sheep gonadotropin, when administered in doses non-stimulating to the intact animal, produced an increase in ovarian weight in the operated littermate. Although augmented ovarian response to pregnant mare serum was noted in the partially nephrectomized rat, the results could be accounted for by the body weight difference between the operated and intact control groups. Pregnancy urine extracts administered in three different doses produced significantly heavier ovaries in the operated rat than in the littermate control. The absolute increase in ovarian weight averaged approximately the same for each dose level covering an eightfold dosage range.—*R.B.G.*

HAMILTON, JAMES.

Male hormone stimulation is prerequisite and an incitant in common baldness. *Am. J. Anat.* 71: 451. 1942.

The etiology of common baldness was studied experimentally in men with testicular insufficiency, namely: 10 eunuchoids; 94 eunuchs, 10 of whom had been castrated prepubertally, 34 bilaterally orchidextomized during adolescence, and 50 castrated as adults. Common baldness never appeared in the 54 men who did not mature sexually. This is scarcely fortuitous in view of the genetic predisposition to alopecia in some of these men as indicated by tendencies to become bald when provided with exogenous androgens and loss in normal men, 43% according to Snyder and Yingling. Concomitantly the amounts of dandruff and sebaceous secretion were less in castrates than in normal men. Male hormone treatment of 12 of these castrate and eunuchoid men, providing stimulation considered equivalent to that in normal men, resulted in increased sebaceous secretions, acne and dandruff, and 4 instances of baldness in a pattern observed in normal men. Progressive loss of head hair ceased when androgenic medication was discontinued in 2 cases, recommenced upon further therapy. Castration of men becoming bald prevented progression of alopecia but promoted no general regrowth of hair on bald areas. Pedigrees of patients receiving testosterone compounds, suggest that a genetic predisposition and male hormone stimulation are necessary to development of common baldness.—*Author's Summary.*

JONES, G. E. S., AND R. W. TE LINDE.

An evaluation of progesterone therapy in treatment of endometrial hyperplasia. *Bull. Johns Hopkins Hosp.* 71: 282. 1942.

Of 28 cases of functional uterine bleeding associated with pathological picture of endometrial hyperplasia treated with progesterone, only 2 required radical procedures. Reduction of incidence of hysterectomy and irradiation therapy from 37 to 7% by use of progesterone indicated value of therapy. Dosage of progesterone and time interval of administration is discussed.—*D.A.M.*

KAHLE, P. J., H. D. OGDEN, JR., AND P. L. GETZOFF.

Effect of diethylstilbestrol and diethylstilbestrol dipropionate on carcinoma of the prostate gland. I. Clinical observations. *J. Urol.* 48: 83. 1942.

Seven cases of adenocarcinoma of the prostate, 6 proven by biopsy, were treated for 2 years with diethylstilbestrol and diethylstilbestrol dipropionate in doses averaging 5 mg. intramuscularly, three times a week. Treatment in all cases has afforded prompt relief of pain and urinary symptoms with general improvement in health. Regression of lesion has occurred in all patients and have lost their malignant characteristics as judged by rectal palpation. Clinical improvement is associated with regression of metastatic lesions to bones and lymph nodes. No side effects of treatment were encountered except for a transient gynecomastia in one patient.—*D.A.M.*

SCHENKEN, J. R., E. L. BURNS AND P. J. KAHLE.

Effect of diethylstilbestrol and diethylstilbestrol dipropionate on carcinoma of the prostate gland. II. Cytological changes following treatment. *J. Urol.* 48: 99. 1942.

See abstract above. Regressive nuclear and cytoplasmic changes occurred in neoplastic cells of all cases. Nuclei showed reduction in size, condensation of chromatin, loss of nucleoli and mitotic figures and pyknosis. Nuclear diameters diminished by 18–56% during treatment. Regressive cytoplasmic changes consisted of appearance of vacuoles, displacement of nuclei toward lumen of acinus, rupture of cell membranes and coalescence of vacuoles.—*D.A.M.*

KEARNS, W. M.

Treatment of carcinoma of the prostate with estrogens. *Wisconsin M. J.* 41: 575. 1942.

Thirty-seven patients ranging from 48 to 86 years (average 67) were treated. Seven died during treatment, 5 of intercurrent causes and 2 in which progress of the carcinoma appeared to be the cause. The estrogen, diethylstilbestrol was administered orally in 1 mg. doses, three times a day for 2 to 3 weeks, the dosage then being reduced to 2 mg. daily for 2 to 4 weeks, and then decreased to 1 mg. daily indefinitely. Clinical improvement was evidenced by gain in weight, usually relief from pain throughout the body, improvement in blood count, slowing of sedimentation rates and an approach to normal in phosphatase estimation. After 4–5 months skeletal roentgenograms improved. The prostate decreased in size and lost its dense nodularity, fixation and indiscrete outline. Gastric and breast stimulation irritation developed in several of the patients and in the recent treatment ethinyl estradiol was substituted for diethylstilbestrol using .05 mg. doses. Several patients were implanted with two 10 mg. pellets of estradiol or two 15 mg. pellets of estradiol benzoate once monthly. Gastric irritation is avoided by this procedure. Administration of estrogen is considered of preference to castration.—*D.A.M.*

MILLER, MARY L.

Neutral steroids in the urine of individuals with benign hypertrophy of the prostate. *J. Urol.* 47: 846. 1942.

Androsterone, trans-dehydroandrosterone (isolated as 3-chloro-Δ^5-androstenone-17) and cholesterol were isolated from neutral steroid fraction of urine of patients with benign prostatic hypertrophy. Comparison of values for normal men and eunuchs indicated somewhat smaller amounts of androsterone but no significant difference for trans-dehydroandrosterone. There was no evidence for the occurrence of abnormal hormone.—*D.A.M.*

MILLER, MARY L., AND R. A. MOORE.

Variation in the daily urinary excretion of androgens in relation to benign hypertrophy of the prostate. *J. Urol.* 48: 544. 1942.

Daily variation in urinary androgen was measured for a period of 30 days in 4 men, ages 73 to 81, 2 men with benign hypertrophy and two of comparable age without prostatic hypertrophy. Daily fluctuations in the two persons with hypertrophy were no greater than in the two without.—*D.A.M.*

NEWERLA, G. J.

The history of the discovery and isolation of the male hormone. *New England J. Med.* 228: 39. 1943.

The history of the discovery and isolation of the androgenic principle of testes is traced. An interesting chronological tabulation and an excellent bibliography are appended.—*R.B.G.*

SELYE, HANS, AND S. ALBERT.

The effect of various steroids in intact male rats. *Am. J. M. Sc.* 204: 876. 1942.

All hormonally active steroids (whether they

be folliculoid, testoid, luteoid, corticoid, or spermatogenic) cause involution of the Leydig cells of the testis. Of these the folliculoids are the most active on a dosage basis. The Leydig cell involution does not result from the direct steroid action but due to the inhibition of the pituitary gonadotropic hormone inhibition the action of the steroids on the structural appearance of numerous other organs has also been tabulated. —*C.P.*

SIMON, S. M., AND A. F. ULLMAN.

A clinical study of 105 patients treated with estrogen and progesterone. *Connecticut State M. J.* 6: 921. 1942.

Eighty-one menopausal patients, natural, surgical and irradiated, were treated once weekly with estradiol dipropionate in doses of 1 mg. for the first week or two followed by 0.2 mg. doses for a total period of 5 or 6 weeks. This was followed by intervals between treatment of 2–4 weeks depending on relief of symptoms. Thirty-nine of the patients received thyroid and sedatives in addition to estrogen therapy. Sixty or 62% of patients had excellent or adequate results, 22 or 27% inadequate and 9 or 11% had no relief. Colored patients seem to respond more favorably than white. Twelve cases of oligomenorrhea treated with estradiol dipropionate (total dose 5 mg.) for 3 weeks followed by progesterone (total dose 6 mg.) for one week. The series was then repeated for an average of 3 months. In 10 or the 12 patients, a normal menstrual flow lasting for several months was attained. Eight cases of secondary amenorrhea also treated with estradiol dipropionate and progesterone yielded only 3 satisfactory results. Three patients with juvenile menorrhagia were relieved and their menstrual cycles completely regulated with progesterone.—*D.A.M.*

HYPOPHYSIS

ANDERSON, J. A., AND WM. R. MURLIN.

Antagonism of pitressin and adrenal cortical extract in human diabetes insipidus. *J. Pediat.* 21: 326. 1942.

The physiological antagonistic action of adrenal cortical extract in pitressin on the excretion of sodium, chloride and water was confirmed in the human subject with diabetes insipidus. Cortical extract fails to increase potassium excretion unless pitressin is supplied. It is suggested that facultative reabsorption of water as induced by pitressin must be in progress before kidney tubules can selectively excrete sodium and potassium.—*D.A.M.*

PANCREAS

ALTSCHUL, A., AND A. NATHAN.

Diabetes mellitus in Harlem Hospital outpatient department in New York. A comparison of certain etiologic factors in Negro and white patients. *Jour. Am. Med. Assoc.* 119: 248. 1942.

The number of new and referred diabetic patients at the Harlem Hospital outpatient department increased 2½ times in the 1935–39 period. In 7 years the mortality increased 76% for Negro as compared with 26% for white diabetic patients. The onset of diabetes was much earlier in the female Negro than in the female white patient. Heredity and familial incidence were factors in the Negro diabetic etiology. Obesity was equally common in both Negro and white female patients. Undernutrition was slightly higher in the Negro than in the white groups. The Negroes had higher per cent in the mild diabetic group than did whites.—Courtesy *Biol. Absts.*

ANDERSON, G. E.

Problem of retinitis in the diabetic patient. *Arch. Ophth.* 28: 679. 1942.

Present-day orthodox treatment of diabetes mellitus has been disappointing in so far as it concerns the prevention of premature arteriosclerosis in general and diabetic retinitis in particular. There seems to be a relationship between the development of fatty infiltration of the liver and a similar process in the arteries—so universal in all diabetics of long duration. Both of these processes are intimately related to faulty fat metabolism. The diabetic should be kept from obligatory fat metabolism by forced carbohydrate and protein metabolism by means of diets relatively high in both moieties and low in fat, together with adequate insulin. Weight loss in the arteriosclerotic diabetic is to be meticulously avoided since it invites unnecessary fat mobilization from the fat depots and amounts to the equivalent of a high-fat diet. Conventional normal readings for blood sugar are not physiologic for the arteriosclerotic diabetic. Their maintenance not infrequently invites relative hypoglycemic states to which there is compensatory suprarenal response and secondary hyper-

tension with resulting retinal hemorrhage. Heroic insulin administration is for the same reason to be deprecated as meddlesome therapy. Weight-reduction by dietary restriction in the well developed arteriosclerotic carries with it considerable hazard of speeding up the underlying vascular degeneration. The problem of weight should be handled prophylactically long before atherosclerosis has had the time necessary for its development.—*Author's Summary.*

BLOCH, F. J.

Retinopathy in juvenile diabetes mellitus. *Arch. Ophth.* 28: 891. 1942.

Retinal changes in juvenile diabetics have been so rarely reported that cases below 35 years of age were considered non-existent. This fact was used to support the theory that diabetic retinopathy does not exist. The well-known fundus changes in diabetic patients were supposed to be caused by hypertension or arteriosclerosis. One case is reported of diabetic retinopathy in a 22 year old girl and one in a 37 year old woman. Blood pressure and renal function were normal. —*Author's Summary.*

BUEDING, E., H. WORTIS AND H. D. FEIN.

Pyruvic acid metabolism in diabetes mellitus. *Am. J. M. Sc.* 204: 838. 1942.

In diabetes mellitus there is little or no increase in blood pyruvate following the ingestion of glucose. If insulin is then administered, a significant rise in blood pyruvic acid occurs.—*C.P.*

SUNDERMAN, F. W.

The water and electrolyte distribution in diabetes mellitus. Dehydration in diabetes. *Am. J. M. Sc.* 205: 102. 1943.

The administration of glucose to the severely diabetic patient (without ketosis) is accompanied by an increase of water, NaCl, and glucose in the circulating plasma. The absolute values increase although the concentration per unit volume may decrease. In depancreatized dogs a continued transfer of water, NaCl and glucose from the tissues into the circulation also occurs. In the diabetic patient the opposite occurs upon the administration of insulin. This action of insulin in readjusting the water and electrolyte partitions is considered important in diabetes.—*C.P.*

WAYBURN, E., AND H. GRAY.

The two-dose glucose tolerance test. *Am. J. M. Sc.* 204: 823. 1942.

This study of the two-dose glucose tolerance test reveals that a close relation exists between blood glucose values and glycosuria in this test and that the 1 hour glucose level is the best single diagnostic aid in diabetes. The two hour blood glucose level aids in differentiating the severer classes of diabetics. Errors due to dietary changes are erased in the two-dose method since the initial dose acts as a stabilizer for variations in the preceding diet.—*C.P.*

THYROID

BRONSTEIN, I. P., L. E. BOWER AND J. MURPHY.

Familial cretinism. Two brothers exhibiting thyroid deficiency and epiphyseal dysgenesis. *Am. J. M. Sc.* 205: 114. 1943.

The rare condition of two brothers, both cretins at the ages of 13 and 23 years respectively, are reported. The family had a history of frequent thyroid trouble. Epiphyseal dysgenesis was present in both brothers. They both responded remarkably well to thyroid therapy.—*C.P.*

GREENE, A. M., AND L. M. HURXTHAL.

Postoperative follow-up study of 469 thyrocardiac patients. *New England J. Med.* 225: 811. 1941.

Four hundred and sixty-nine thyrocardiac cases in which operation was performed from 1922 to 1937 have been followed. In the first group of 303 patients operated on between 1922 and 1932, 164, or 54.1 per cent, are living; 122 have normal heart rhythm, and 42 continue to have auricular fibrillation. Of the second group of 166 operated cases, 128 patients are still living, 98 of whom have normal heart rhythm. The case mortality was 4.5 per cent, and the operative mortality was slightly less than 3 per cent. The incidence of recurrent hyperthyroidism in the first group followed from six to sixteen years was over 10 per cent, which shows that the incidence increases the longer the period after operation. Although these patients are restored to normal activity for the most part, their expectancy of life based on the present follow-up falls considerably short of the predicted expectancy.—*Author's Summary.*

SOLEY, M. H., AND R. S. STONE.

Roentgen ray treatment of hyperthyroidism. *Arch. Int. Med.* 70: 1002. 1942.

Forty-three patients with unquestioned hyperthyroidism were treated by roentgen therapy to the thyroid. One half of these (22 patients) received iodine, while the other half (21 patients) received no iodine in conjunction with the roentgen ray treatment. Twenty-five patients (or 58.1 per cent) were clinically free of hyperthyroidism in an average time of 8.7 months from the onset of treatment and 8 (or 18.6 per cent) were markedly improved. Both groups had a coincident decrease in the size of the thyroid to within normal limits. The average basal metabolic rate was plus 35.7 per cent before treatment and minus 2 per cent after treatment. Three patients were inadequately followed, and their exact status is unknown. Two patients with congestive heart failure, which had been present for 2 and 4 years respectively prior to treatment, died before their hyperthyroidism could be "cured." Four patients were operated on because of failure of roentgen ray therapy and for other reasons. One patient deliberately had been given a single course as preparation for a subtotal thyroidectomy. Complications consisted of tracheitis and esophagitis in 15 patients. In order to avoid these complications, the authors changed the method of therapy. Radiation to the thyroid now is given through 1 port anteriorly. The authors conclude to the effect that roentgen ray therapy in adequate dosage is successful in bringing about subtotal destruction of the thyroid in patients with toxic diffuse goiter. In the majority it results in relief in clinical symptoms, fall of basal metabolic rate to a normal level and decrease of the thyroid to normal size.—*I.B.*

STEWART, H. J., AND W. F. EVANS.

Peripheral blood flow in myxedema. *Arch. Int. Med.* 69: 808. 1942.

Using a method which Stewart and Jack and Stewart and Evans have employed in earlier studies, the authors made measurements of peripheral blood flow in 6 patients suffering from myxedema when they were first seen before treatment and again on several occasions during the course of thyroid therapy. In addition, certain other measurements of the circulation were recorded. The results are summarized as follows: In the myxedematous state the peripheral blood flow was decreased. During the administration of thyroid, a progressive increase in peripheral blood flow occurred, so that a linear relation was maintained. The cardiac output is decreased in patients with myxedema. How the organs share this disease is not known, but it is now shown that the amount of blood allotted to the periphery is decreased. The circulation time before treatment was prolonged. During treatment progressive decreases took place. Shortening of circulation time roughly ran parallel to increase in basal metabolic rate and in peripheral blood flow. The changes in pulse rate and pulse pressure followed roughly the increases in basal metabolic rate and peripheral blood flow. For the most part, change in the average skin temperature followed the changes in basal metabolic rate and in peripheral blood flow. Conservation of heat has been suggested as an explanation for the decrease in peripheral blood flow in untreated patients suffering from myxedema.—*I.B.*

TROTTER, W. R., AND K. C. EDEN.

Localized pretibial myxedema in association with toxic goitre. *Quart. J. Med.* 11: 229. 1942.

The literature on localized myxedema is reviewed; and it is pointed out that the condition, while fairly familiar to dermatologists, is rarely discussed in the general medical literature. The most striking feature of the condition is that no case of localized pretibial myxedema has been described in a patient without past or present thyrotoxicosis. Of 69 cases assembled by the authors the time of onset of the pretibial myxedema was prior to treatment of thyrotoxicosis in 32, after thyroidectomy in 35 and after x-ray therapy in 2. In 11 of the 35 cases which followed thyroidectomy, the thyrotoxicosis had either recurred or had not been entirely abolished by the operation at the time the pretibial myxedema occurred. The lesions tend to disappear spontaneously over a period of a few years. No form of therapy yet employed has seemed to affect the condition. The authors point out that pituitary overactivity is probably present in both toxic goitre and hypothyroidism and, consequently, might be the cause of mucin deposition in the tissues in these 2 conditions. They emphasize that such a theory is wholly unsupported by any direct evidence but suggest that some of the facts brought out in the article could be explained in this way. For example, "localized pretibial myxedema is constantly associated with the disease toxic goitre, and yet cannot be the direct product of thyroid toxemia. The high incidence of recurrent thyrotoxicosis suggests that the skin lesions are particularly liable to occur in patients

in whom the stimulus to thyroid overaction is unusually powerful or persistent. The occasional simultaneous development of exophthalmos and localized pretibial myxedema could be explained by such a hypothesis."—*J.E.H.*

TROTTER, W. R. AND N. WALLACE.

Thyroxine as a practical treatment of myxedema. *Brit. M. J.* 1: 183. 1942.

A typical case of myxedema is described. The patient reacted to the oral administration of thyroid with vomiting, edema of the face and pains in the limbs. Thyroxin was administered intravenously with good therapeutic results; the maintenance dose was found to be 7.5 mg. given in a single injection once every four weeks.—*E.B.A.*

WATSON, C. J., D. CRAIG AND N. BEACH.

Myxedematous ascites. *Internat. Clin.* 4: 177. 1941.

This is a brief discussion of the subject with 2 illustrative case reported in detail. In 1 of these, hydrothorax occurred, but this is not believed to be due to the myxedema. Ascites without heart failure occurs in myxedema only occasionally. Of 22 cases of myxedema, observed over a period of 20 years, but 2 cases of this type were observed. The presence of mucin in the ascetic fluid in myxedema indicates that the ascites is directly related to the underlying cellular disturbance of the disease, i.e., elaboration of an abnormal fluid rich in mucin.—*I.B.*